ENHANCEMENT OF WILD LYCOPERSICON GERMPLASM FOR RESISTANCE TO BEMISIA ARGENTIFOLII (HOMOPTERA: ALEYRODIDAE)

By

SAMUEL GAITHURU MUIGAI

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Abstract of Dissertation Presented to the Graduate School
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ENHANCEMENT OF WILD LYCOPERSICON GERMPLASM FOR RESISTANCE TO BEMISIA ARGENTIFOLII (HOMOPTERA: ALEYRODIDAE)

By

Samuel Gaithuru Muigai

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Major Department: Horticultural Science

Accessions of wild Lycopersicon germplasm were screened for resistance to the silverleaf whitefly (SLWF), Bemisia argentifolii Bellows & Perring. Various levels of resistance were found among accessions of L. pennellii (Corr.) D'Arcy, L. hirsutum f. typicum Humb. & Bonpl. and L. hirsutum f. glabratum C. H. Mull. None of the Lycopersicon peruvianum (L.) Mill. and L. pennellii var. puberulum Phil. accessions were resistant.The correlation between the resistance and the densities of glandular trichomes was low, negative and often significant for types IV and I, but mostly positive for type VI trichomes. Therefore, trichome density might not be a reliable criteria when selecting for resistance to the SLWF in a breeding program.

Resistant plants from the different species differed in the chemical profiles of their glandular trichome exudates as revealed by Thin Layer Chromatography (TLC), and Gas Chromatography (GC). Profiles also differed among accessions and among plants within accessions. L. pennellii possessed acylsugars which were composed of acylsucroses and acylglucoses. L. hirsutum f. typicum had sesquiterpenoids, whereas methyl ketones were found in L. hirsutum f. glabratum. These chemicals were lacking or were in low concentrations in susceptible accessions.

Different interspecific crosses were made with the objective of combining different resistance chemistries for a better source of SLWF resistance in breeding programs. F_1 hybrids between resistant L. pennelli and L. hirsutum f. typicum were resistant, whereas hybrids between L. pennellii and the cultivated tomato, L. esculentum, were moderately resistant to susceptible. Hybrids between L. hirsutum and tomato were mainly susceptible. The chemical profiles of the F_1 revealed by TLC had novel components. The composition of the acylsugars was also different from that found in the parents, with most F_1s having a higher proportion of acylsucroses. The presence of the sesquiterpenoids in the F_1s was suspected in some of the hybrid plants but was difficult to confirm through TLC analysis. The profiles of hybrids between the L. hirsutum and the tomato did not possess any main components, whereas some hybrids of the tomato and L. pennellii possessed acylsugars but at low concentration.

The F_1s were self-incompatible. Segregation for resistance occurred in L. pennellii X L. hirsutum f. typicum full-sib F_2 populations.

L. pennellii, L. hirsutum f. glabratum, and L. hirsutum f. typicum exhibited antixenosis and antibiosis mechanisms of resistance to the silverleaf whitefly. The effects of these resistant genotypes on the SLWF were mechanically transferable by

appressing the trichome exudates onto the leaves of the susceptible tomato, indicating an association between the factors mediating the resistance and the glandular trichomes.

The methyl ketones, 2-tridecanone and 2-undecanone, had repellent, fumigant, and contact toxicity to the SLWF, whereas 2-pentadecanone had contact toxicity only. Ginger oil (composed of sesquiterpenoids) also had the three insecticidal effects on the SLWF.

These findings support the feasibility of combining the different resistance chemistries which would likely provide multiple mechanisms of resistance which might be more difficult for the SLWF to overcome.

CHAPTER 1
INTRODUCTION

The tomato (Lycopersicon esculentum Mill.) is one of the world's important food crops. In many countries, it is ranked second only to potatoes (Solanum tuberosum L.) as a popular vegetable (Purseglove, 1974). Global production increased rapidly within the past century to nearly 50 million MT (Tigchelaar, 1986). Tomato is versatile in usage. The per capita consumption is approximately 25 kg in the United States both as fresh and processed product.

The silverleaf whitefly (SLWF), Bemisia argentifolii Bellows & Perring, is one of the major arthropod pests that attack and damage a tomato crop directly and indirectly. A severe outbreak of this pest in Florida first occurred in 1986. The outbreak initially was attributed to a new strain of the sweetpotato whitefly (SPWF), Bemisia tabaci Gennadius, but the whitefly has since been designated the SLWF, a new species (Bellows et al., 1994).

The silverleaf whitefly causes injury by feeding on the plant sap and thereby weakening the host plant severely (Schuster et al., 1996). Adults and nymphs excrete abundant honeydew, which is deposited on the upper surfaces of plant parts and serves as a substrate for sooty mold. Produce that has been soiled by sooty mold has reduced aesthetic and economic value. Irregular ripening in tomato is a disorder that is associated with SLWF (Schuster et al., 1990). The SPWF transmits various plant viruses including: tomato yellow leaf curl virus (Cohen and Litzany, 1966; Berlinger and Dahan, 1987; Cohen and Harpaz, 1964; Kisha, 1984), tomato leaf curl virus (Yassin and Nour, 1965), tomato pale chlorosis disease (Cohen and Antignus, 1982), tomato necrotic dwarf virus (Duffus, 1987), and tomato golden mosaic (Anon., 1983). The SLWF most likely vectors the same range of viruses and some new viruses including, the newly described tomato mottle virus (ToMoV) (Polston et al., 1993).

Schuster et al.(1989) attributed at least $125 million annual loss by tomato growers in the state of Florida to direct yield reduction and SLWF control expenses. The SLWF has become a serious pest in the hot-dry climate of southern California and southern Texas and has caused large economic losses (Perring et al., 1991, 1993).

The silverleaf whitefly is difficult to control with insecticides because feeding and oviposition occur mainly on the lower surfaces of leaves (Sharaf, 1986), and because the SLWF develops resistance to insecticides applied for its control. The advantages of growing resistant cultivars are their compatibility with other components of integrated pest management, conservation of the environment, and slow development of insecticide resistance.

No commercial tomato cultivar has been reported to be resistant to the SLWF. Kisha (1981) observed that an L. esculentum accession with greater trichome density also had decreased SPWF oviposition and a higher ratio of females trapped on the leaves. On the basis of this observation, the conclusion was that trichomes could contribute to SPWF resistance in tomato. Butler and Henneberry (1991) reported differences in the number of SPWF adults attracted to different commercial tomato cultivars. Heinz and Zalom (1995) observed higher rate of oviposition by the SLWF on tomato cultivars with high trichome density. Limited information is available on the existence of SPWF and/or SLWF resistance among the germplasm of wild species of the Lycopersicon genus. Berlinger et al. (1983) found some Lycopersicon hirsutum f. glabratum and Lycopersicon pennellii accessions to be resistant to SPWF. Resistance to the SPWF has been identified in some accessions of L. hirsutum f. glabratum (Shevach-Urkin, 1983) and L. hirsutum f. typicum (Yorit, 1986). De Ponti et al. (1990) reported resistance to the SPWF in some accessions of L. hirsutum f. typicum, L. hirsutum f. glabratum and L. pennellii. According to Berlinger and Dahan (1987), some ecotypes of L. pennellii possess a very high degree of resistance to SPWF. Appreciable levels of resistance have been observed in some accessions of L. pennellii; however, the evaluation of the resistance varies when tested by different methods and under different conditions (D. J. Schuster, pers. comm.).

The identification and measurement of plant resistance to insects requires reliable and accurate screening techniques. The results of a bioassay can be affected by insect variables, plant variables, and environmental variables (Smith et al., 1994). Shevach-Urkim (1983) used potted intact plants and caged detached leaves from glasshouse raised L. hirsutum f. glabratum accessions in choice and no-choice experiments to assay for resistance. Resistance to SPWF was identified in the accessions PI 134418 and PI 129157. As a quick and simple alternative to the bioassay screening method, stickiness of the leaves caused by trichome exudates was estimated by measuring the amount of sugar granules that stuck to the leaf (Berlinger et al., 1984). Eight L. hirsutum f. typicum accessions were screened by Yorit (1986) for resistance to SPWF by use of insect bionomics criteria and viral sensitivity in the field. The criteria used to define resistance were ovipositional preference, survival, fecundity and the amount of honeydew secretion. When compared to the cultivated tomato, only two accessions, LA 1777 and IVT 771498, were resistant with respect to all parameters.

Resistance of wild Lycopersicon spp. to whiteflies and other arthropods is often related to trichomes. The types of trichomes that occur in the genus Lycopersicon were described by Luckwill (1943). The main trichome differences between the cultivated tomato and the wild taxa L. hirsutum f. glabratum, L. hirsutum f. typicum and L. pennellii are the presence of type IV glandular trichomes exclusively in the wild species and presence of type V non-glandular trichomes in the cultivated species. Shevach-Urkin (1983) reported that whiteflies became entangled and were physically immobilized in the sticky exudate of glandular trichomes of some L. hirsutum f. glabratum accessions. Yorit (1986) reported similar results for some L. hirsutum f. typicum germplasm but found no relationship between the level of entanglement and the number and type of glandular trichomes. Both investigators attributed the main mechanism of resistance in their materials to physical interference by the glandular trichomes. They found no correlation between the then recognized leaf volatile chemicals and the resistance.

Resistance to a number of arthropod pests.has been associated with allelochemicals found in some Lycopersicon spp. (Anon., 1975; Lin et al., 1987; Steffens and Walters, 1991; Kennedy et al., 1981; 1992). Soost et al., (1968) obtained leaf-oils from several Lycopersicon spp. by steam distillation of whole foliage. Gas-liquid-chromatography (GLC) was used to separate individual components that were identified by comparing retention times, infrared (IR) spectra, and nuclear magnetic resonance (NMR) spectra with pure standards. Major components were 11, 12, and 13 carbon methyl ketones. The trichome exudates of L. hirsutum f. glabratum had 26% of 2-undecanone and 68% of 2-tridecanone, whereas the trichome exudates of L. hirsutum f. typicum had 71% of the ketone 2-dodecanone. L. esculentum trichome exudates consisted of 30% of 2-tridecanone.

Lin et al.(1987) analyzed isooctane extracts from glandular heads of type VI trichomes of L. esculentum, L. hirsutum f. typicum, and L. hirsutum f. glabratum. Major chemical constituents were identified and quantified by gas chromatography and mass spectrometry. Two-undecanone, 2-tridecanone and one unknown sesquiterpene accounted for approximately 95% of the contents of the glands of the L. hirsutum f. glabratum accession PI 134417. An accession of L. hirsutum f. typicum (LA 361) had two unknown sesquiterpenes which accounted for 6% of the gland content and were insecticidal to the tomato pinworm (Keiferia lycopersicella (Wals.)) and the beet armyworm (Spodoptera exigua (Hubner)). Contrary to Soost et al.(1968), Lin et al.(1987) did not detect 2-tridecanone in glandular heads of L. esculentum or 2-dodecanone in L. hirsutum f. typicum. The major volatiles in type VI glandular trichome secretion of the L. hirsutum f. typicum accession PI 251303 and several others of this botanical form were the sesquiterpenes zingiberene and gamma-elemene (Weston et al., 1989). Methyl ketones (11C and 13C) dominate the composition of type VI trichome exudates of L. hirsutum f. glabratum. Guo et al. (1993) found volatiles of L. hirsutum f. typicum to be more repellent to mites compared to L. hirsutum f. glabratum. Snyder et al.(1993) found that the repellent action of some accessions of L. hirsutum f. typicum could be partially attributed to a unique terpene, 2,3-dihydrofarnesoic acid. At the same time, they found that some highly repellent accessions were totally devoid of this sesquiterpenoid.

Hawthorne et al.(1992) demonstrated antixenotic resistance in the L. pennellii accession PI 246502 to the leafminer, Liriomyza trifolii (Burgess), and determined 2,3,4-tri-o-acylglucoses as the principal allomones. Resistance of the accession LA 716 to the potato aphid, Macrosiphum euphorbiae (Thomas), has also been attributed to the same chemistry (Goffreda et al., 1990). According to Weston et al. (1989) and Guo et al. (1993), the profiles of volatiles from type I, IV, or VI glandular trichomes of a particular accession of a botanical form of L. hirsutum are similar to each other, and to that of the whole leaf wash. They suggest that volatiles in hexane leaflet washes contain mainly compounds associated with trichomes.

Factors that affect trichome density, and the level of allelochemicals, indirectly affect the expression of resistance in Lycopersicon. Good and Snyder (1988) reported significant seasonal (summer/winter) effects on the density of type IV, V, and VI trichomes and the concentration of zingiberene and gamma-elemene in the F2 of an interspecific cross between cultivated tomato and L. hirsutum f. typicum. During summer, the levels of 2-tridecanone and 15C compounds in trichome exudates were higher and so was mite survival. Significant photoperiod effects on the trichome density in L. esculentum, L. hirsutum f. typicum, and L. hirsutum f. glabratum, and a significant interaction between photoperiod and both genotype and trichome type was observed (Snyder and Hyatt, 1984). The density of type IV trichomes was higher under short day conditions. Under short day conditions, trichome density and zingiberene content were higher and dependent on temperature in the L. hirsutum f. typicum accession PI 126445 (Gianfagana et al., 1992). Temperature had no effect on trichome density and zingiberene content under long-day conditions. The L. hirsutum f. glabratum accession PI 134417 produced more 2-tridecanone and inflicted higher mortality on tobacco hornworm when grown under long days relative to short days (Kennedy et al., 1981). Light intensity had no effect either on the ketone levels or resistance, but the day length by light intensity interaction affected the density of glandular trichomes. Shevach-Urkin (1983) found that high light intensity influenced the level of resistance in PI 365907, a L. hirsutum f. glabratum accession, in a positive manner, although, overall, this accession was susceptible. Leaf expansion affects trichome density. Plant age, gland age, and location of the leaf on the plant canopy affect the chemical composition of the glandular trichome exudates (Lin et al., 1987).

In crosses of L. pennellii PI 246502 and L. esculentum, Lemke and Mutschler (1984) reported that the presence of type IV trichomes was governed by 2-duplicate dominant epistatic genes, whereas the density of the same trichome type was inherited quantitatively with a high broad sense heritability. Snyder and Carter (1985) observed trichome types I, IV, V, and VI and VII in F_1 hybrids of L. hirsutum (PI 252303) x L. esculentum. Fery and Kennedy (1987) reported that the resistance to the tobacco hornworm (Manduca sexta L.) was conditioned by at least three recessive genes in this accession.

The SLWF is a species that differs from the earlier known SPWF (Perring et al., 1993) upon which the available information on resistance is based. Therefore, the primary purpose of this research was to screen wild Lycopersicon germplasm for resistance to the SLWF, to investigate the chemical profiles of exudates from glandular trichomes of the resistant genotypes, to enhance the resistance by combining different resistance chemistries in single genotypes, and to investigate the mechanism(s) of the resistance. It is envisaged that silverleaf whitefly resistance based on multiple chemistries and mechanisms of resistance will be more amenable for use in tomato breeding programs and might be durable.

CHAPTER 2
GREENHOUSE AND FIELD SCREENING OF WILD LYCOPERSICON GERMPLASM FOR RESISTANCE TO THE SILVERLEAF WHITEFLY, BEMISIA ARGENTIFOLII BELLOWS & PERRING

Introduction

The silverleaf whitefly (SLWF), Bemisia argentifolii Bellows & Perring, formerly sweetpotato whitefly (SPWF) biotype "B", is the key pest of tomato in south Florida (Schuster et al., 1996) and resulted in losses of 500 million dollars to US agriculture in 1992 (Perring et al:, 1993). This pest is difficult to control with insecticides because feeding and oviposition occur mainly on the lower surfaces of leaves (Sharaf, 1986) and because whiteflies develop resistance to insecticides applied for thier control (Dittrich and Ernest, 1990; Stansly et al., 1991). Growing resistant cultivars would be advantageous because they are compatible with other components of integrated pest management, conserve the environment, and slow the development of insecticide resistance. Little research has been reported on the resistance of commercial tomato cultivars or any germplasm within the cultivated species of tomato (Lycopersicon esculentum Mill.). Butler (1991) tested a limited number of commercial tomato cultivars and reported small differences in the oviposition preference of the sweetpotato whitefly (SPWF), B. tabaci (Gennadius). Heinz and Zalom (1995) observed higher oviposition rate by the SLWF on tomato cultivars with high trichome density. Limited information is available on the resistance of wild Lycopersicon to the SPWF and/or SLWF. Resistance to SPWF has been reported in some accessions of Lycopersicon hirsutum f. glabratum C. H. Mull., and Lycopersicon hirsutum f. typicum Humb. & Bonpl. (Berlinger et al., 1983; Shevach-Urkin, 1983; Yorit, 1986; De Ponti et al., 1990). According to these reports, resistance was achieved by physical interference, viz., the adult whiteflies became entangled in the sticky glandular trichomes resulting in high mortality. Appreciable levels of resistance have been observed in some accessions of L. pennellii (Corr.) D'Arcy (Barten et al., 1995); however, the evaluation of the resistance varies when tested by different methods and under different conditions. Generally, resistance can be found provided that a wide array of germplasm is tested (de Ponti et al., 1990).

Researchers have searched for resistance to greenhouse whitefly and SPWF with some success. In spite of these findings, the transfer of resistance to L. esculentum has not been successful (Schuster et al., 1996), and currently no commercial cultivars with resistance to the whitefly are available. As a result, no breeding lines are available that possess resistance to this insect (J. W. Scott, pers. comm.). Since the SLWF is a species that differs from the earlier known SPWF (Perring et al., 1993) upon which the available information on resistance is based, it is necessary to screen wild Lycopersicon germplasm for resistance to the SLWF. The objective of this research was to evaluate resistance to SLWF in selected wild Lycopersicon accessions with artificial infestation under greenhouse conditions and under natural infestation in the field in an attempt to find diverse resistance components. It was anticipated that the degree of success in transferring and retaining adequate levels of resistance might be influenced by its source and that different sources may provide different resistance components.

Materials and Methods

Greenhouse Screening

Seeds of the wild Lycopersicon accessions were acquired from the Genetic Resources unit of the United States Department of Agriculture (USDA) Plant Introduction Station at Geneva, New York, and from the Tomato Genetic Stock Center (TGSC) at Davis, California. Thirty-two accessions of wild Lycopersicon germplasm were first evaluated in a greenhouse choice bioassay. The criteria for including an accession in this initial evaluation included reported resistance to arthropod pests and/or reported chemical constituents of glandular trichomes (Berlinger et al., 1983; Yorit, 1986; Lin et al., 1987; Weston et al., 1989; King et al., 1990; Hawthorne et al., 1992; Channayapa, 1992; Guo et al., 1993). There were 8 accessions of Lycopersicon pennellii (Corr.) D'Arcy and 2 of the subspecies L. pennellii var puberulum Phil; 8 and 10 accessions of L. hirsutum f. glabratum C. H. Mull. and L. hirsutum f. typicum Humb. & Bonpl., respectively; and 4 accessions of Lycopersicon peruvianum (L.) Mill. For each accession, twenty-five seedlings were raised in 128-celled (34 cm^3 cell size) Speedling^R flats containing Jiffy^R soil mix. At the third leaf stage of growth, eight plants were chosen randomly and transplanted into 1500 cm^3 pots containing a standard soil mixture. Eight plants of the susceptible tomato cv. Solar Set were grown as a control. The pots were placed on benches in water-cooled, insect proof fiber glass greenhouses and were watered as necessary. Supplemental lighting was provided using 40-watt cool white fluorescent and 300-watt incandescent light bulbs to obtain a 16:8 (L:D)h photoperiod and 120-126 microeinsteins $m^{-2}s^{-1}$ (Li-cor Quantum Meter, Model LI-185A, Lambda Inst. Corp., Lincoln, Neb.) light intensity at one meter below the lights. The lights were suspended above the plants and were raised as the plants grew. Temperature in the greenhouses ranged from 17-26 ^{0}C and the air remained humid. The plants were pruned to one stem.

Approximately 21-28 days after transplanting, the plants were mass infested with SLWF from a colony maintained in the laboratory on the dwarf tomato cv. Florida Lanai (Augustine et al., 1981).

The numbers of whitefly adults, eggs, 1st-4th instars, and empty pupal cases (emerged adults) were determined on two occasions. The first evaluation was completed 32 days after mass infestation of the greenhouse when the plants had approximately ten true leaves. Four leaflets proximal to the terminal leaflet on the leaves at nodes 5 and 7 (counting from the plant apex, starting with the first unfolded leaflet) were harvested, using a pair of steel forceps to sever them from the plant and transfer them directly into a 15-cm diameter plastic petri dish. The samples were taken to the laboratory and whitefly adults, eggs, crawlers, and nymphs and empty pupal cases were counted on each leaflet surface for all 8 leaflets using a dissecting microscope (Wild-Heerbrugg, Heerbrugg, Switzerland) at a magnification of 12X. The second whitefly count was done 45 days after mass infestation. In this second round of assessment (data not presented), the plants were subjectively rated for resistance based on the relative infestation of foliage of all ages and locations in the plant canopy. Complete absence of all insect lifestages (highly resistant) was assigned a score of 1. Where any degree of infestation was observed, the four lateral leaflets proximal to the terminal one on the leaflet on the node designated as number 6 at the time of the first evaluation were excised, taken to the laboratory and evaluated for SLWF as above. At the time of this subjective scoring, node 6 had aged further and attained node 8-9 status. A score of 5 (highly susceptible) was assigned when counts of SLWF individuals of all lifestages was more than 100. Plants with a total of 1-10, 11-50, and 51-100 SLWF were respectively assigned a score of 2 (resistant), 3 (moderately resistant), and 4 (susceptible).

The density of type IV, V, and VI trichomes was determined from the average of counts made at four binocular microscope fields on each surface of the pair of lateral leaflets proximal to the terminal leaflet of the fifth node that were sampled at 32 days after mass infestation. One of the leaflets was used to determine trichome density on the adaxial leaflet surface, whereas the other was used for the abaxial surface. The dissecting microscope set at 50X was used for these counts. The fields of view, located in the interior middle section of the leaflet and with equal number on each side of the midvein, measured approximately 7.1 mm^2 each. The area of the leaflets used for insect counts and trichome density determination was measured by use of a portable area meter (LI-COR model LI-3000, Lambda Instruments Corporation, USA).

Field Resistance Verification

Based on the evaluation results in the primary greenhouse screening, 33 plants selected from 22 accessions were tested in the field to verify the resistance that was observed in the initial greenhouse screening. The selected plants had been scored from highly resistant (score 1) to moderately (score 3) resistant in both the primary and secondary greenhouse evaluations. These resistant plants, and four susceptible plants representing each of the wild Lycopersicon species evaluated, were cut back to a stem height of approximately 30 cm. Two grams of fertilizer (N:P:K; 20:20:20) dissolved in water at 4 g/l was then added to each pot and multiple lateral shoots allowed to grow. These shoots were used to vegetatively propagate the selected plants. The cuttings from the lateral shoots were rooted under intermittent mist.

The rooted cuttings described above were transplanted in the field at the Gulf Coast Research and Education Center, Bradenton, Florida in March, 1995. There were 38 entries consisting of the 33 resistant test plants, 4 apparently susceptible wild plants and two plants of the susceptible tomato cv. Solar Set. Except for the tomato cultivar that was raised from seed, the other test materials had been obtained by vegetative propagation. The experiment was laid out in 3 randomized complete blocks with each entry represented by one plant per block. The plants were set on 84 cm wide by 20 cm high beds of EauGallie fine sand. The beds were spaced on 150 cm centers with seepage irrigation ditches spaced 6 beds apart. The blocks were arranged parallel to the beds and the irrigation ditches. The plants were spaced at 91 cm with 2 m separating blocks within the bed. Standard growing practices were applied (Howe et al., 1994). Bacillus thuringiensis was applied to control lepidopteran insect pests but no other insecticides were used.

Natural SLWF infestation was allowed to occur. Whitefly and trichome density measurements were completed 90 days after the cuttings were transplanted into the field. Leaf samples were taken on the major shoot(s) and brought to the laboratory. The procedures followed for leaf sampling, insect counts, and trichome density determinations were identical to those described for the primary evaluation in the greenhouse.

To compare genotypes, the mean whitefly densities and trichome densities were transformed (square root of (X + 0.5)) prior to analysis of variance (ANOVA) using PROC GLM statement of SAS and means separation by the Duncan's Multiple Range Test. Spearman correlations between trichome densities and insect densities were calculated using the CORR statement of SAS (SAS Institute Inc., 1988).

Results

Greenhouse Screening

The density of whiteflies differed significantly among accessions (Appendix A-1). Differences among accessions weresignificant for the density of eggs, crawlers, 2nd and 3rd instars, and dead adults for adaxial and abaxial leaf surfaces. Densities of pupae on the adaxial surface, exuviae and live adults on both leaf surfaces were not significantly different among accessions. Whitefly densities on the adaxial leaf surfaces (Table 2-1) were comparatively lower than on the abaxial surface (Table 2-2).

In general, densities of total whitefly, and immature lifestages were lowest for the accessions of Lycopersicon pennellii, followed by L. hirsutum f. typicum, and L. hirsutum f. glabratum. The accessions of Lycopersicon peruvianum and L. pennellii var puberulum screened in this experiment had the highest densities of all whitefly lifestages.

Variation of whitefly density among individual plants of the same accession was observed even within accessions which had relatively low mean insect density. This within-accession variability was consistent during both occasions when the density of whiteflies was assessed first by absolute counts and later by the subjective method.

Trichome density differences among accessions were highly significant (Appendix A-2) for both leaf surfaces. There was a wide range of densities within Lycopersicon species. Type IV and type VI trichomes were present on the foliage of the wild Lycopersicon taxa (Table 2-3) except for two accessions of L. peruvianum. Among the L. peruvianum accessions, LA 364 and LA 2151 had type V and VI but no type IV, whereas PI 127830 and LA 1292 possessed types IV, V, and VI. One L. hirsutum f. typicum accession, PI 127826 had type V trichomes in addition to the glandular types. Accession PI 199381, a L. hirsutum f. glabratum, did not have type

Table 2-1. Mean density(cm^{-2}) of silverleaf whitefly on the adaxial surface of the 7th leaf node on plants of different accessions of wild Lycopersicon germplasm evaluated in greenhouse multiple-choice experiment to screen for resistance to Bemisia argentifolli - Fall 1994.

Taxa and accession number	Number of plants	Eggs	Crawlers	2nd & 3rd instars	Pupae	Exuviae	Live adults	Dead adults
L. pennellii								
LA 1302	8	<0.1 b	0.2 bc	0.1 c	<0.1 b	<0.1 ab	<0.1 ab	1.0 b
LA 1340	8	0.1 b	0.1 c	<0.1 c	0 b	0 b	0 b	2.0 a
LA 1674	5	0.3 b	<0.1 c	<0.1 c	<0.1 b	0 b	0 b	2.0 a
LA 1809	7	0.2 b	0.1 c	0.1 c	<0.1 b	0 b	<0.1 ab	0.2 bc
PI 246502	8	0.6 b	<0.1 c	0.3 bc	0 b	0 b	<0.1 a	0.6 bc
LA 2560	8	<0.1 b	0.1 c	<0.1 c	0 b	0 b	0 b	0.5 bc
LA 716	6	0 b	0 c	<0.1 c	0 b	0 b	0 b	0.4 bc
LA 2963	8	0.9 b	0.2 bc	<0.1 c	<0.1 b	<0.1 b	<0.1 ab	0.3 bc
L. pennellii var. puberulum								
LA 1911	8	1.6 b	1.4 b	0.2 bc	<0.1 b	<0.1 b	<0.1 b	0.3 bc
LA 1912	2	9.4 a	5.7 a	2.2 a	0.1 ab	0 b	0 b	0 c
L. hirsutum f. glabratum								
LA 1223	8	0.7 b	0.2 bc	0.1 b	0.1 ab	0 b	<0.1 b	<0.1 c
PI 126449	8	0.7 b	0.1 bc	<0.1 c	0 b	0 b	<0.1 ab	<0.1 c
LA 2144	8	1.1 b	0.1 bc	<0.1 c	0.1 ab	0 b	<0.1 b	0.1 c
LA 1265	8	0.3 b	0.1 c	0.1 bc	<0.1 b	0 b	0 b	<0.1 c
PI 134417	8	0.4 b	0.1 c	<0.1 c	0 b	0 b	<0.1 b	0.1 c
PI 134418	8	0.6 b	<0.1 c	<0.1 c	<0.1 b	0 b	<0.1 b	0.1 c
PI 199381	8	1.0 b	0.4 bc	0.2 bc	<0.1 b	0 b	<0.1 b	<0.1 c
PI 251305	8	1.1 b	0.2 bc	0.1 c	0 b	0 b	<0.1 b	<0.1 c
L. peruvianum								
PI 127830	8	1.6 b	0.4 bc	0.7 b	0.2 a	0 b	0 b	0 c
LA 1292	8	1.7 b	0.2 bc	<0.1 c	0 b	0 b	0 b	<0.1 c
LA 2151	8	1.7 b	0.9 bc	0.1 c	<0.1 b	0 b	0 b	<0.1 c
LA 364	8	1.8 b	0.2 bc	0.1 c	0 b	0 b	0 b	0 c

Table 2-1 -- continued

Taxa and accession number	Number of plants	Eggs	Crawlers	2^{nd} & 3^{rd} instars	Pupae	Exuviae	Live adults	Dead adults
L. hirsutum f. typicum								
PI 126445	5	0.5 b	0.1 bc	<0.1 c	0 b	0 b	0 b	<0.1 c
PI 127826	7	0.1 b	0.1 c	<0.1 c	<0.1 b	0 b	0 b	0.1 c
PI 127827	8	0.7 b	0.2 bc	0.2 bc	0.1 ab	0 b	0 b	0.4 bc
LA 1353	8	0.2 b	<0.1 c	<0.1 c	0 b	0 b	0 b	0.1 c
LA 1772	8	0.2 b	<0.1 c	<0.1 c	0 b	0 b	0 b	0.1 c
LA 1777	8	0.1 b	0 c	<0.1 c	0 b	0 b	0 b	0.5 bc
LA 1927	8	1.3 b	<0.1 bc	0.1 c	<0.1 b	0 b	0 b	0.1 c
LA 2155	8	0.8 b	0.1 c	0.1 bc	0 b	0 b	0 b	0.2 c
LA 386	8	0.4 b	0.1 c	<0.1 c	0.1 b	0 b	0 b	0.1 c
PI 390513	8	0.6 b	0.2 bc	0.1 c	<0.1 b	<0.1 b	0 b	0.1 c
L. esculentum								
Solar Set	8	0.8 b	0.5 bc	0.4 bc	0.1 ab	0.1 a	0 b	<0.1 c

Means within a column followed by the same letter are not significantly different ($p \le 0.05$) by Duncan's Multiple Range Test.

Statistical analysis performed on square root transformed data but means are original data.

Table 2-2. Mean density (cm^{-2}) of silverleaf whitefly on the abaxial surface of the 7th leaf node on plants of different accessions of wild Lycopersicon germplasm evaluated in greenhouse multiple-choice experiment to screen for resistance to Bemisia argentifolii - Fall 1994.

Taxa and accession number	Number of plants	Eggs		Crawlers		2^{nd} & 3^{rd} instars		Pupae		Exuviae		Live adults		Dead adults		All * lifestages	
L. pennellii																	
LA 1302	8	1.6	g-i	1.1	c-f	0.5	d-g	0.1	bc	0	b	0	b	0.5	b	1.9	d-g
LA 1340	8	<0.1	hi	<0.1	f	<0.1	g	0	c	<0.1	b	<0.1	b	1.7	a	0.1	g
LA 1674	5	4.6	d-h	0.6	c-f	0.3	e-g	0	c	<0.1	b	<0.1	b	2.0	a	2.0	c-g
LA 1809	7	0.4	hi	<0.1	ef	0.8	c-g	0.1	bc	0	b	0	b	0.1	cd	0.9	e-g
PI 246502	8	0.5	g-i	0.2	d-f	<0.1	g	0.1	bc	<0.1	b	<0.1	b	0.4	bc	0.8	e-g
LA 2560	8	0.5	g-i	0.5	c-f	0.1	fg	0	c	<0.1	b	<0.1	b	0.3	bc	0.6	fg
LA 716	5	0	i	0	f	0	g	0	a-c	0	b	0	b	0.3	b-d	<0.1	g
LA 2963	8	10.0	b-i	1.0	c-f	0.4	d-g	0.2	a-c	<0.1	ab	<0.1	ab	0.2	b-d	6.3	b-g
L. pennellii var. puberulum																	
LA 1911	8	18.3	a-c	6.6	b	2.5	b-e	0.7	a-c	0	b	0	b	0.1	cd	15.6	b
LA 1912	2	27.1	a	20.4	a	8.1	a	0.7	a-c	<0.1	a	<0.1	a	0	d	36.8	a
L. hirsutum f. glabratum																	
LA 1223	8	11.9	b-f	3.6	b-f	2.0	b-g	0.8	a-c	0	b	0	b	<0.1	d	9.7	b-d
PI 126449	8	3.0	d-i	0.6	c-f	0.5	c-g	0.1	bc	0.1	bc	0	b	0.1	cd	2.5	c-g
LA 2144	8	19.6	ab	4.0	b-d	1.8	b-f	0.6	a-c	<0.1	ab	<0.1	ab	0	d	13.9	b
LA1265	8	2.9	d-i	0.6	c-f	0.6	c-g	0.1	bc	0	b	0	b	<0.1	cd	2.3	c-g
L. pennellii																	
PI 134417	8	0.6	g-i	0.1	ef	<0.1	g	0	c	0	b	0	b	0.1	cd	0.5	e-g
PI 134418	8	0.3	hi	<0.1	f	<0.1	g	<0.1	c	0	b	0	b	0.1	cd	0.5	e-g
PI 199381	8	8.7	b-h	3.0	b-e	1.0	b-g	0.2	a-c	0	b	0	b	0	d	7.3	b-e
PI 251305	8	11.9	b-f	1.6	b-f	1.6	b-g	0.3	a-c	<0.1	b	<0.1	b	0	d	8.3	b-d

Table 2-2 -- continued

Taxa and accession number	Number of plants	Eggs		Crawlers		2^{nd} & 3^{rd} instars		Pupae		Exuviae		Live adults		Dead adults		All * lifestages	
L. peruvianum																	
PI 127830	8	7.5	b-i	2.2	b-e	3.7	bc	1.1	ab	0	b	0	b	0	d	8.7	b-f
LA 1292	8	8.5	b-h	1.0	c-f	0.3	d-g	0	c	0	b	0	b	0	d	5.9	b-g
LA 2151	8	5.2	c-i	1.8	b-f	0.7	c-g	0.1	bc	0	b	0	b	0	d	5.2	b-g
LA 364	8	12.3	b-e	1.2	c-f	0.6	c-g	0	c	0	b	0	b	0	d	8.0	b-d
L. hirsutum f. typicum																	
PI 126445	5	3.3	b-i	0.8	b-f	0.4	c-g	0.2	a-c	0	b	0	b	<0.1	d	2.6	b-g
PI 127826	7	1.5	f-i	0.4	c-f	0.5	c-g	<0.1	bc	<0.1	b	<0.1	b	0.5	cd	1.3	d-g
PI 127827	8	5.6	c-i	2.2	b-f	1.4	b-g	1.5	a	0	b	0	b	0.1	b-d	6.1	b-g
LA 1353	8	1.2	g-i	0.1	ef	<0.1	g	0	c	0	b	0	b	<0.1	cd	0.8	e-g
LA 1772	8	3.2	d-i	0.5	c-f	0.6	c-g	0.1	bc	0	b	0	b	<0.1	cd	2.3	c-g
LA 1777	8	1.0	g-i	0.4	c-f	0.1	fg	0.3	a-c	0	b	0	b	0.2	b-d	1.0	e-g
LA 1927	8	18.5	b-e	1.4	b-f	0.5	c-g	0.1	bc	0	b	0	b	<0.1	cd	9.6	bc
LA 2155	8	13.8	b-d	2.4	b-f	1.8	b-g	0.4	a-c	0	b	0	b	<0.1	cd	9.6	bc
LA 386	8	2.0	e-i	0.6	c-f	0.4	c-g	<0.1	c	0	b	0	b	0.1	b-d	1.7	c-g
PI 390513	8	8.8	b-g	4.0	b-d	2.5	b-d	0.8	a-c	0	b	0	b	<0.1	cd	8.4	b-d
L. esculentum																	
Solar Set	8	5.5	b-i	2.9	b-e	3.9	b	0.7	a-c	<0.1	b	<0.1	b	0	d	7.5	b-d

Means within a column followed by the same letter are not significantly different ($p \leq 0.05$) by Duncan's Multiple Range Test. Statistical analysis performed on square root transformed data but means are original data.

* Total immatures + exuviae averaged over total leaf surface area.

Table 2-3. Mean trichome density (cm^{-2}) for the 5th leaf node on plants of different accessions of wild Lycopersicon germplasm evaluated in greenhouse multiple-choice experiment to screen for resistance to Bemisia argentifolii - Fall 1994.

Taxa and accession number	Number of plants	Trichome type					
		Adaxial surface			Abaxial surface		
		IV	V	VI	IV	V	VI
L. pennellii							
LA 1302	8	464 e-g	0 d	54 k-m	1043 d-h	0 d	16 k
LA 1340	8	1050 a-d	0 d	66 k-m	1692 a-e	0 d	35 k
LA 1674	5	851 b-e	0 d	104 j-l	1152 d-h	0 d	19 k
LA 1809	8	1265 a-c	0 d	104 j-l	1650 a-f	0 d	22 k
PI 246502	8	1605 a	0 d	27 k-m	3029 a	0 d	12 k
LA 2560	8	1312 ab	0 d	116 jk	1730 a-e	0 d	42 k
LA 716	6	1081 a-d	0 d	26 k-m	1455 b-g	0 d	11 k
LA 2963	8	1019 a-d	0 d	88 j-m	1464 b-g	0 d	7 k
L. pennellii var. puberulum							
LA 1911	8	321 f-h	0 d	132 i-k	782 f-h	0 d	16 k
LA 1912	2	223 g-i	0 d	12 i-m	498 g-i	0 d	10 k
L. hirsutum f. glabratum							
LA 1223	8	32 ij	0 d	376 e-h	2548 ab	0 d	205 ij
PI 126449	8	44 ij	0 d	701 b-d	1174 c-h	0 d	790 a
LA 2144	8	33 ij	0 d	129 i-k	2938 a	0 d	60 k
LA 1265	8	41 ij	0 d	454 d-g	2368 a-c	0 d	473 c-g
PI 134417	8	99 h-j	0 d	699 b-d	2190 a-c	0 d	764 ab
PI 134418	8	191 g-j	0 d	691 b-d	2667 a-c	0 d	812 a
PI 199381	8	0 j	34 d	642 b-e	388 ij	1660 c	375 e-h
PI 251305	8	16 ij	0 d	278 g-i	2253 a-e	0 d	144 j

Table 2-3 -- continued

Taxa and accession number	Number of plants	Trichome type					
		Adaxial surface			Abaxial surface		
		IV	V	VI	IV	V	VI
L. peruvianum							
PI 127830	8	283 h-j	2230 d	72 k-m	419 ij	3377 b	60 k
LA 1292	8	115 ij	414 c	283 g-i	307 ij	1456 c	194 j
LA 2151	8	0 j	2261 d	228 h-j	0 j	5028 a	253 h-j
LA 364	8	0 j	454 c	363 f-h	0 j	2399 b	324 g-i
L. hirsutum f. typicum							
PI 126445	5	91 h-j	0 d	668 b-d	362 hi	0 d	612 a-e
PI 127826	7	693 d-f	173 d	808 bc	1728 b-g	367 d	469 d-g
PI 127827	8	762 c-f	0 d	607 b-f	1658 a-f	0 d	480 c-g
LA 1353	8	1475 ab	0 d	926 ab	2560 a-c	0 d	669 a-c
LA 1772	8	592 c-f	0 d	764 bc	1023 d-h	0 d	772 ab
LA 1777	8	1311 ab	0 d	1105 a	2305 a-d	0 d	675 a-c
LA 1927	8	309 f-h	0 d	405 e-h	1004 e-h	0 d	640 a-d
LA 2155	8	121 h-j	0 d	521 c-f	1243 c-h	0 d	355 f-i
LA 386	8	600 c-f	0 d	670 b-d	1721 a-e	0 d	546 b-f
PI 390513	8	5 ij	0 d	822 ab	1661 b-g	0 d	600 a-d
L. esculentum							
Solar Set	8	0 j	781 b	288 g-i	0 j	2207 bc	222 h-j

Means within a column followed by the same letter are not significantly different ($p \leq 0.05$) by Duncan's Multiple Range Test. Statistical analysis performed on square root transformed data, but means are original data.

IV trichomes on the adaxial leaf surface and had trichome type V on both leaf surfaces. Trichome density variation within accessions was also apparent.

Spearman correlation coefficients (r) were calculated between trichome densities and whitefly density measurements. The coefficients were generally low (Table 2-4). The correlation coefficients between the density of type IV and insect density were negative and significant ($P<0.001$) for eggs, crawlers, and 2nd and 3rd instars. A low, positive and significant correlation was obtained for the same insect immatures as above and type V trichome density. Correlation between the density of dead adults on adaxial and abaxial surfaces and type IV trichomes on the adaxial surface of the leaves was positive, moderate and significant. This insect variable had a small negative but significant correlation with the density of type V and type VI trichomes.

Field Resistance Verification

The analysis of variance and mean separation for the field experiment was by plant rather than by accession since the objective was to verify the results obtained from the greenhouse resistance screening study. For the data obtained from the abaxial leaf surface, significant differences existed among the plants for the density of eggs, crawlers, 2nd and 3rd instars, and pupae (Appendix A-3). Density of crawlers on the adaxial surface differed significantly ($P<=0.05$) but the differences for the other insect

Table 2-4. Correlation (r) between the densities of B. argentifolii lifestages on the 7th leaf node, and the density of trichome types on the 5th leaf node of plants evaluated in greenhouse experiment to screen accessions of wild Lycopersicon germplasm for resistance to the silverleaf whitefly - Fall 1994.

Whitefly lifestage and leaf surface	Trichome type and leaf surface					
	Adaxial			Abaxial		
	IV	V	VI	IV	V	VI
Adaxial						
Eggs	-0.35***	0.21***	0.01ns	-0.20***	0.22***	0.03ns
Crawlers	-0.39***	0.33***	-0.07ns	-0.28***	0.33***	-0.06ns
2nd & 3rd instars	-0.31***	0.23***	-0.15**	-0.28***	0.22***	-0.16*
Pupae	-0.17**	0.12*	-0.05ns	-0.12ns	0.11ns	-0.067ns
Exuviae	-0.04ns	-0.01ns	-0.05ns	0.01ns	-0.01ns	-0.11ns
Live adults	0.07ns	-0.11ns	-0.14*	0.11ns	-0.11ns	-0.15*
Dead adults	0.59***	-0.41***	-0.16**	0.26***	-0.42***	-0.20**
Abaxial						
Eggs	-0.52***	0.23***	0.02ns	-0.28***	0.24***	0.01ns
Crawlers	-0.45***	0.27***	0.0ns	-0.25***	0.27***	0.0ns
2nd & 3rd instars	-0.40***	0.17**	-0.06ns	-0.21***	0.15*	-0.06ns
Pupae	-0.27***	0.12ns	0.01ns	-0.02ns	0.12ns	-0.02ns
Exuviae	0.06ns	-0.11ns	-0.17**	0.04ns	-0.11ns	-0.17**
Live adults	0.06ns	-0.11ns	-0.17**	0.04ns	-0.11ns	-0.17**
Dead adults	0.55***	-0.36***	-0.24***	0.22***	-0.36***	-0.26***

248 observations used in computing each correlation coefficient value.

ns, *, **, *** = Nonsignificant (ns) or significant at 0.05(*), 0.01(**), and 0.001(***) level.

stages were non-significant. Differences among plants for the density of dead adults on the leaf surface were non-significant for either surface. No live adults were found stuck on the leaf surfaces.

None of the plants of L. pennellii had any whiteflies, either adult or immature stages (Table 2-5). This degree of consistency among plants was not observed for the other taxa represented in the experiment. One plant from the accession LA 1265 of L. hirsutum f. glabratum was the most densely infested by whiteflies of all lifestages and even surpassed the susceptible controls.

Differences among the tested plants were highly significant ($P<0.001$) for the density of all the trichome types evaluated and both leaf surfaces (Appendix A-4). Among the wild species, type V trichomes were exclusively found in L. peruvianum plants, which also lacked type IV (Table 2-6). The plants from the other taxa had both type IV and type VI at varying proportions relative to each other. Correlation coefficients between the densities of various trichomes were low but varied in the level of significance. The density of type IV trichomes had a negative but significant correlation with the density of eggs, crawlers and the 2nd and 3rd instars (Table 2-7). Type V trichome density had a low, positive and mostly significant correlation with the density of whitefly stages. Type VI had a small positive and significant correlation with the density of eggs on the abaxial leaf surface.

Discussion

Greenhouse Screening

Differences in the density of silverleaf whitefly were highly significant for the combined total of all insect immature stages (Table 2-1). Considered separately, the differences in the density of eggs, crawlers, 2nd and 3rd instar nymphs, and pupae (4th instar) were highly significant ($P<0.001$). A large number of the accessions had lower density of whiteflies than the susceptible tomato cv. Solar Set, which was used as a standard (Table 2-2). Some accessions were as susceptible as the commercial cultivar, whereas some others were more susceptible than the standard. Adopting the density of the insect on the host plant as an index of relative resistance, it is apparent that the accessions screened exhibited wide variation for resistance to the SLWF.

Considerable variation for resistance was observed within some accessions (data not presented). This variation was consistently observed on both occasions where the plants were scored for whitefly density. The wild species under study are largely self-incompatible (Hogenboom, 1972) and outcrossing. Consequently, this intra accession variation could be due to segregation resulting from earlier cross pollination between genotypes of different resistance levels. This phenomenon could influence the outcome when screening these wild Lycopersicon germplasm as a source of resistance to pests, and the remedy might be that a sizeable number of plants need to be screened for each accession.

Table 2-5. Mean density (cm^{-2}) of silverleaf whitefly on both surfaces of the 7th leaf node on plants of Lycopersicon germplasm originally selected from different accessions screened in the greenhouse and presently evaluated in the field to verify resistance to Bemisia argentifolii - Spring 1995.

Taxa and accession number	Selected plant identity	Eggs	Crawlers	2nd & 3rd instars	Pupae	Total insects
L. pennellii						
LA 1340	p46	0 b	0 d	0 d	0 c	0 d
LA 1340	p66	0 b	0 d	0 d	0 c	0 d
LA 1340	p76	0 b	0 d	0 d	0 c	0 d
LA 1340	p86	0 b	0 d	0 d	0 c	0 d
LA 716	p67	0 b	0 d	0 d	0 c	0 d
LA 716	p37	0 b	0 d	0 d	0 c	0 d
PI 246502	p21	0 b	0 d	0 d	0 c	0 d
PI 246502	p51	0 b	0 d	0 d	0 c	0 d
PI 246502	p71	0 b	0 d	0 d	0 c	0 d
LA 1302	p15	0 b	0 d	0 d	0 c	0 d
LA 1674	p210	0 b	0 d	0 d	0 c	0 d
LA 1674	p410	0 b	0 d	0 d	0 c	0 d
LA 1674	p810	0 b	0 d	0 d	0 c	0 d
LA 2560	p14	0 b	0 d	0 d	0 c	0 d
LA 2963+	p62	1.0 b	4.3 d	2.7 cd	0 c	8.0 d
L. hirsutum f. typicum						
LA 386	t121	2.7 b	0.3 d	0 d	0 c	3.0 d
LA 386	t621	1.3 b	0.7 d	0 d	0 c	2.0 d
LA 1353	t411	0 b	0 d	0 d	0 c	0 d
LA 1353	t511	3.3 b	0.7 d	0 d	0 c	4.0 d
LA 1353	t711	0 b	0 d	0 d	0 c	0 d
LA 1772	t222	7.3 b	3.0 d	1.0 d	0 c	11.3 cd
LA 1777	t212	2.7 b	1.3 d	0 d	0 c	4.0 d
LA 1777	t612	18.3 b	1.3 d	2.7 cd	0 c	22.3 b-d
LA 1927	t613	22.3 b	6.7 d	0.3 d	0 c	27.3 bc
LA 2155+	t617	11.3 b	4.7 d	1.0 d	0 c	17.0 b-d
PI 127826	t230	3.0 b	1.3 d	0 d	0 c	4.3 d
PI 127826	t630	0 b	0 d	0 d	0 c	0 d
PI 127827	t429	0 b	0 d	0 d	0 c	0 d
PI 127827	t529	2.3 b	3.3 d	0 d	0 c	5.7 d
L. hirsutum f. glabratum						
LA 2144+	g816	16.7 b	20.0 bc	6.0 bc	0 c	42.7 bc
LA 1265	g515	101.3 a	45.7 a	17.0 a	3.3 a	167.3 a

Table 2-5 -- continued

Taxa and accession number	Selected plant identity	Eggs	Crawlers	2^{nd} & 3^{rd} instars	Pupae	Total insects
PI 126449	g631	9.7 b	0.3 d	0 d	0 c	10.0 d
PI 134417	g227	1.7 b	0 d	0 d	0 c	1.7 d
L. peruvianum						
LA 2151	r320	4.7 b	7.0 cd	0.7 d	0 c	12.3 cd
LA 2151	r520	1.0 b	1.3 d	1.0 d	0 c	3.3 d
PI 127830+	r128	3.0 b	10.5 b-d	1.5 d	0 c	15.0 cd
PI 127830	r328	2.0 b	1.7 d	1.0 d	0 c	4.7 d
L. esculentum						
Solar Set+	esc	16.0 b	23.0 b	7.8 b	0.8 b	47.7 b

Means within a column, followed by the same letter are not significantly different ($p \leq 0.05$) by Duncan's Multiple Range Test.

Statistical analysis performed on square root transformed data but means are original data.

Total insects = eggs + crawlers + 2^{nd} & 3^{rd} instars + pupae

+ treatment included as a susceptible control.

Table 2-6. Mean trichome density (cm^{-2}) for the 5th leaf node on plants of wild Lycopersicon germplasm originially selected from different accessions screened in the greenhouse and presently evaluated in the field to verify resistance to Bemisia argentifolii - Spring 1995.

Taxa and accession number	Selected Plant Identity	Trichome type					
		Adaxial Surface			Abaxial Surface		
		IV	V	VI	IV	V	VI
L. pennellii							
LA 1340	p46	275 kl	0 d	35 jk	359 k	0 f	9 i-k
LA 1340	p66	435 h-l	0 d	14 k	541 h-k	0 f	20 g-k
LA 1340	p76	419 i-l	0 d	38 i-k	954 g-j	0 f	40 g-k
LA 1340	p86	384 j-l	0 d	18 k	463 i-k	0 f	25 g-k
LA 716	p67	890 d-i	0 d	13 k	968 g-i	0 f	21 g-k
LA 716	p37	805 e-j	0 d	39 i-k	968 g-i	0 f	14 g-k
PI 246502	p21	531 g-k	0 d	24 k	964 g-i	0 f	12 h-k
PI 246502	p51	664 f-k	0 d	30 jk	1032 f-h	0 f	12 h-k
PI 246502	p71	786 e-j	0 d	33 k	1004 f-h	0 f	5 k
LA 1302	p15	309 kl	0 d	20 k	531 h-k	0 f	19 g-k
LA 1674	p210	386 j-l	0 d	51 i-k	533 h-k	0 f	20 g-k
LA 1674	p410	410 j-l	0 d	39 i-k	448 jk	0 f	21 g-k
LA 1674	p810	324 kl	0 d	9 i-k	463 i-k	0 f	16 g-k
LA 2560	p14	333 kl	0 d	18 k	469 i-k	0 f	19 g-k
LA 2963	p62	82 m-o	0 d	12 k	108 i	0 f	5 k
L. hirsutum f. typicum							
LA 386	t121	2001 a	0 d	148 gh	4015 a	0 f	161 e-j
LA 386	t621	1487 a-d	0 d	688 a	3548 ab	0 f	593 a-c
LA 1353	t411	2080 ab	0 d	681 a	2707 bc	0 f	631 ab
LA 1353	t511	1353 b-e	0 d	231 e-h	2063 cd	0 f	468 b-d
LA 1353	t711	885 d-h	0 d	609 ab	1959 c-e	0 f	131 e-i

Table 2-6 -- continued

Taxa and accession number	Selected Plant Identity	Trichome type					
		Adaxial Surface			Abaxial Surface		
		IV	V	VI	IV	V	VI
LA 1772	t222	1310 c-e	0 d	272 e-h	1703 d-f	0 f	471 b-e
LA 1777	t212	1525 a-e	0 d	215 e-h	1924 c-e	0 f	324 c-f
LA 1777	t612	1038 c-g	0 d	418 b-e	1038 g-i	0 f	96 f-k
LA 1927	t613	161 l-n	0 d	290 e-g	547 h-k	0 f	898 a
LA 2155	t617	9 o	0 d	167 f-h	6 l	0 f	270 d-f
PI 127826	t230	1106 c-f	0 d	227 e-h	2264 cd	0 f	603 ab
PI 127826	t630	1043 c-g	0 d	576 a-c	2249 cd	0 f	322 b-e
PI 127827	t429	1576 a-c	0 d	376 c-e	1872 c-e	0 f	523 a-d
PI 127827	t529	224 lm	0 d	500 a-d	1230 e-g	0 f	385 b-e
L. hirsutum f. glabratum							
LA 2144	g816	426 i-l	0 d	131 g-i	1261 e-g	0 f	90 f-k
LA 1265	g515	15 no	0 d	195 f-h	1660 d-g	0 f	148 e-h
PI 126449	g631	36 m-o	0 d	246 e-h	298 k	0 f	214 e-g
PI 134417	g227	184 lm	0 d	316 d-f	1849 c-e	0 f	149 e-g
L. peruvianum							
LA 2151	r320	0 o	1207±196 b	41 i-k	0 l	4338 b	19 g-k
LA 2151	r520	0 o	994±7 b	35 jk	0 l	5018 a	11 i-k
PI 127830	r128	0 o	1872±276 a	7 k	0 l	3461 c	6 jk
PI 127830	r328	0 o	2120±257 a	14 k	0 l	2843 d	5 k
L. esculentum							
Solar Set	esc	0 o	645±94 c	130 h-j	0 l	2151 e	58 g-k

Means within a column, followed by the same letter are not significantly different ($p \leq 0.05$) by Duncan's Multiple Range Test. Statistical analysis performed on square root transformed data but means are original data.

Table 2-7. Correlation (r) between the densities of B. argentifoliii lifestages on the 7th leaf node, and the density of trichome types on the 5th leaf node plants of wild Lycopersicon germplasm originally selected from different accessions screened in the greenhouse and presently evaluated in the field to verify resistance to the silverleaf whitefly - Spring 1995.

Whitefly lifestage and leaf surface	Trichome type and leaf surface					
	Adaxial			Abaxial		
	IV	V	VI	IV	V	VI
Adaxial						
Eggs	-0.19*	0.20*	0.04ns	-0.03ns	0.14ns	0.12ns
Crawlers	-0.39***	0.34***	0.05ns	-0.31***	0.33***	0.10ns
2nd & 3rd instars	-0.28**	0.47***	-0.13ns	-0.28**	0.47***	-0.21*
Pupae	-0.14	0.23ns	0.08ns	-0.14ns	NC	-0.03ns
Exuviae	NC	NC	NC	NC	NC	NC
Live adults	NC	NC	NC	NC	NC	NC
Dead adults	-0.12ns	0.06ns	-0.09ns	-0.15ns	0.07ns	-0.05ns
Abaxial						
Eggs	-0.31***	0.12ns	0.19*	-0.12ns	0.09ns	0.25**
Crawlers	-0.47***	0.45***	0.05ns	-0.27**	0.43***	0.02ns
2nd & 3rd instars	-0.40***	0.35***	0.02ns	-0.28**	0.33***	0.01ns
Pupae	-0.32***	0.26**	0.08ns	-0.12ns	0.20**	0.09ns
Exuviae	NC	NC	NC	NC	NC	NC
Live adults	NC	NC	NC	NC	NC	NC
Dead adults	-0.03ns	0.04ns	0.03ns	0.01ns	0.05ns	0.02ns

113 observations used in computing each correlation coefficient value.

ns, *,**,*** = nonsignificant(ns), or significant at 0.05(*), 0.01(**), and 0.001(***) level.

NC = not calculated

The highest level of resistance was observed in L. pennellii accessions LA 716, LA 1340 and LA 2560. Other pennellii accessions that were more resistant than 'Solar Set' were LA 1809 and PI 246502 (which is supposed to be equivalent to LA 716). LA 1302 and LA 1674 had some plants that were slightly susceptible. The L. pennellii accession LA 2963 was as susceptible as the cultivated tomato, whereas the L. pennellii var. puberulum accessions LA 1911 and LA 1912 were more susceptible than the control.

It has been reported that L. pennelli LA 716 showed resistance to the potato aphid (Gentile and Stoner, 1968), the greenhouse whitefly and the two-spotted spider mite (Gentile et al., 1968; 1969). In our study, LA 716 and the other accessions listed above were resistant to the SLWF. The resistant plants from the L. pennellii taxon were characterized by the absence of immature stages of the whitefly and some dead and/or live adults trapped in trichome exudates. This would suggest similarity of the components of resistance in the accessions. LA 1340 and LA 1674 had significantly higher numbers of dead, stuck whiteflies compared to all the other accessions (Table 2-1). This might be due to a separate component of resistance present only in these two accessions and not in the other accessions of L. pennellii.

Among the accessions of L. hirsutum f. typicum, LA 2155 and PI 390513 were more susceptible than the tomato cultivar. PI 126445 and LA 1927 were nearly as susceptible as the control, and PI 127827 was moderately resistant. The accessions LA 1772, LA 386 and PI 127826 had some plants with high resistance. The most resistant L. hirsutum f. typicum accessions were LA 1777 and LA 1353. In comparison to L.

pennellii, within accession variability for resistance was more frequent in typicum spp. Schuster (1977) found resistance to the tomato pinworm (Keiferia lycopersicella (Walsingham)) in PI 127826 and PI 134418. Carter et al. (1989) reported resistance of PI 126445 to the colorado potato beetle (Leptinotarsa decemlineata (Say)). Eigenbrode and Trumble (1993) found LA 1777 to be resistant to the beet armyworm (Spodoptera exigua (Hubner)), whereas Juvik et al. (1982) found this accession to be resistant to four lepidopteran pests of tomato in Israel. Fery and Kennedy (1987) found PI 127826 to be susceptible to the tobacco hornworm (Manduca sexta (L.)). Channarayappa et al. (1992) reported lack of transmission of tomato leaf curl virus under field conditions and attributed this to the resistance of LA 386, LA 1777, and PI 390513 to whiteflies.

The L. hirsutum f. glabratum plants were more homogeneous within each accession, but none showed very high resistance. LA 2144 was the most susceptible L. hirsutum f. glabratum accession, but LA 1223 and PI 251305 were also more susceptible than the tomato 'Solar Set'. Accession PI 199381 was about as susceptible as the control. PI 126449 and LA 1265 were more resistant than the control, whereas PI 134417 and PI 134418 were the most resistant f. glabratum accessions evaluated. LA 134417 has been reported resistant to several insect species (Sorenson et al., 1989). Eigenbrode and Trumble (1993) found PI 199381 to be susceptible to the beet armyworm, and they stated that this accession might have been misclassified and actually be a L. hirsutum f. typicum. In our study, it was observed that the glandular heads of the type IV trichomes in this accession appeared to be disintegrating and thereby probably affecting the level of resistance. Consequently, this accession might be undesirable as a source of resistance to the SLWF. In addition, type IV trichomes whose glandular heads have disintegrated might erroneously be classified as type V.

The L. peruvianum accessions were as susceptible as the cultivated tomato. Channarayappa et al. (1992) had observed that the accession PI 127830 did not develop virus symptoms to which it was susceptible. They attributed this to resistance to the whitefly which vectors the virus.

Of the wild species evaluated, L. peruvianum was the only one that consistently had type V trichomes (Table 2-3). This is in agreement with the description of trichomes of Lycopersicon by Luckwill (1943). However, PI 127830 and LA 1292 had type IV trichomes which was unexpected for the species L. peruvianum. It is not possible to explain this anomaly other than to speculate on possible past crossing with a species that possesses type IV trichomes, or the natural presence of type IV trichomes in this species contrary to Luckwill (1943). Type IV trichomes were absent on the leaves of PI 127830 which was grown in the field (Table 2-6).

The density of type IV trichomes was consistently higher than that of type VI in the wild germplasm. The correlation coefficients between trichome density and the density of whiteflies were low but significant in some cases (Table 2-4). The implication for tomato breeding is that trichome density is not a reliable selection criterion for resistance to SLWF. Tomato breeders have been experiencing this dilemma when they attempt to select among segregating material on the basis of glandular trichome density (J. W. Scott, pers. comm.).

Field Resistance Verification

The results for the plants evaluated in the field verification were in good agreement with the primary greenhouse screening results. A few test plants had a significant change. The selected resistant plant from LA 1265 was susceptible in the field (Table 2-5) and plants from LA 1777 appeared more susceptible in the field than in the greenhouse. It is known that interactions can occur between genotypes and the environment and some resistance attributes in Lycopersicon (Gianfagana et al., 1992; Good and Snyder,1988). Trichome density was lower in the field experiment (Table 2-6) for all the test plants. The correlation between trichome and whitefly densities was low but significant in some relationships (Table 2-7). This result is similar to the one obtained in the greenhouse screening experiment.

A sustainable silverleaf whitefly management strategy in tomatoes will most likely combine several techniques into an integrated pest management program. Host plant resistance could have an important role; and, therefore, the need exists to search for reliable resistance that can eventually be introgressed into commercial cultivars. The screening approach used in the research reported here was systematic. Diverse materials were screened by exposing them to high pest pressure and eventually were tested under normal production conditions. The identified resistant germplasm should serve tomato cultivar development through the provision of a broad potential resistance base.

CHAPTER 3
INVESTIGATION OF THE MAIN CHEMICAL CONSTITUENTS OF GLANDULAR TRICHOMES EXUDATES OF SOME WILD LYCOPERSICON GERMPLASM, AND THEIR F_1 INTERSPECIFIC HYBRIDS, RESISTANT TO BEMISIA ARGENTIFOLII

Introduction

Resistance to a number of arthropod pests has been associated with allelochemicals found in some Lycopersicon spp. (Anon., 1975; Lin et al., 1987; Steffens and Walters, 1991; Kennedy et al., 1981; 1992). Soost et al. (1968) obtained leaf oils from several Lycopersicon spp. by steam distillation of intact foliage. Gas-liquid-chromatography (GLC) was used to separate components that were identified by comparing retention times and infra-red (IR) and nuclear magnetic resonance (NMR) spectra with known standards. Major components were 11, 12, and 13 carbon methyl ketones. The distillates of L. hirsutum f. glabratum consisted of 26% 2-undecanone and 68% of 2-tridecanone, whereas the distillates of L. hirsutum f. typicum had 71% of the ketone 2-dodecanone. L. esculentum trichome exudates consisted of 30% of 2-tridecanone.

Lin et al. (1987) analyzed isooctane extracts from glandular heads of type VI trichomes of L. esculentum, L. hirsutum f. typicum, and L. hirsutum f. glabratum. Major chemical constituents were identified and quantified by gas chromatography and mass spectrometry. Approximately 95% of the contents of a gland of the L. hirsutum f.

glabratum accession PI 134417 were comprised of 2-undecanone, 2-tridecanone and one unknown sesquiterpene. An accession of L. hirsutum f. typicum (LA 361) had two unknown sesquiterpenes that were insecticidal to tomato pinworm (Keiferia lycopersicella (Wals.)) and beet armyworm (Spodoptera exigua (Hubner)) and accounted for 6% of the gland content. Contrary to Soost et al.(1968), Lin et al. (1987) did not detect 2-tridecanone in glandular heads of L. hirsutum f. typicum or L. esculentum. Coates et al. (1988) isolated and identified three sequiterpene acids (santalenoic acid and alpha and beta forms of bergamotenoic acid) from hexane extracts of whole leaves of the L. hirsutum f. typicum accession LA 1777. They found that the acids stimulated oviposition in Helicoverpa zea (Boddie). The major volatiles in trichome secretions of the L. hirsutum f. typicum accession PI 251303, and several others of this botanical form, were the sesquiterpenes zingiberene and gamma-elemene (Weston et al., 1989). Methyl ketones (11C and 13C) dominated the composition of trichome exudates of L. hirsutum f. glabratum. Guo et al. (1993) found volatiles of L. hirsutum f. typicum to be more repellent to mites compared to those from L. hirsutum f. glabratum. Snyder et al.(1993) found that the repellent action of some accessions of L. hirsutum f. typicum to two-spotted spider mites (Tetranychus urticae Koch) could be partially attributed to a unique sesquiterpenoid, 2,3-dihydrofarnesoic acid. At the same time, they found that some highly repellent accessions were totally devoid of this sesquiterpenoid.

According to Weston et al. (1989) and Guo et al. (1993), the profiles of volatiles from a type I, IV, or VI glandular trichome of a particular accession of L.

hirsutum were similar to each other and to that of the whole leaflet wash. They suggested that volatiles in leaflet hexane washes contain mainly compounds associated with glandular type IV and VI trichomes.

Fery and Kennedy (1987) studied two crosses: PI 134417 (L. hirsutum f. glabratum resistant to the tobacco hornworm (Manduca sexta (L.))) crossed with cultivated tomato and PI 134417 crossed with PI 127826 (tobacco hornworm susceptible L. hirsutum f. typicum). They found at least three recessive genes conditioned high resistance to tobacco hornworm and that the same genetic system conditioned high 2-tridecanone concentration, which was said to impart the resistance.

Hawthorne et al. (1992) demonstrated antixenotic resistance in the Lycopersicon pennellii accession PI 246502 to the leafminer (Liriomyza trifolii (Burgess)) and determined 2,3,4-tri-o-acylglucoses as the principal allomones. Resistance of accession LA 716 (suspected to be equivalent to PI 246502) to the potato aphid (Macrosiphum euphorbiae (Thomas)) also has been attributed to the same chemistry (Goffreda et al., 1990). Liedl et al. (1995) reported reduced settling and oviposition by the silverleaf whiteflies on tomato leaf surfaces treated with purified acylsugars obtained from trichome exudates of L. pennellii accession LA 716.

Shapiro et al. (1994) found significant differences among accessions of L. pennellii for acylsugars and fatty acid profiles of the acylsugars and for uniformity of acylsugar characteristics among individuals within an accession.

The wild tomato species secrete some allelochemicals that have adverse effects on some insect pests; and therefore, are potential sources of resistance. The cultivated tomato (Lycopersicon esculentum Mill.) neither secretes such chemicals (Fobes et al., 1985) nor does it have type IV trichomes (Snyder and Carter, 1985). The objective of this research was to investigate the main constituents of the glandular trichomes exudates (as revealed by differences in Thin Layer Chromatography (TLC) and Gas Chromatography (GC) chemical profiles) of 1) selected wild Lycopersicon germplasm, 2) F_1 interspecific hybrids of the wild germplasm, and 3)investigate the relationship between these differences in profiles and resistance to the silverleaf whitefly (SLWF)(Bemisia argentifolii Bellows & Perring).

Materials and Methods

Parental Accessions and Plants Used in Primary Screening

The experimental materials included 32 accessions of wild Lycopersicon: eight L. pennellii (Corr.) D'Arcy (LA 1302, LA 1340, LA 1674, LA 1809, PI 246502, LA 2560, LA 716, and LA 2963); two L. pennellii var. puberulum Phil. (LA 1911 and LA 1912); eight L. hirsutum f. glabratum C. H. Mull. (LA 1223, PI 126449, LA 2144, LA 1265, PI 134417, PI 134418, PI 199381, PI 251305); ten L. hirsutum f. typicum Humb. & Bonpl. (PI 126445, PI 127826, PI 127827, LA 1353, LA 1772, LA 1777, LA 1927, LA 2155, LA 386, and PI 390513); and four L. peruvianum (L.) Mill. (PI 127830, LA 1292, LA 2151, and LA 364). The 32 accessions listed above were investigated for resistance to SLWF and for differences in composition of their trichome secretions as revealed by TLC and GC. This study was conducted at the Gulf

Coast Research and Education Center (GCREC), Bradenton, Florida, USA, starting from the fall 1994 season. Analysis of the composition of the exudates was done at the University of Kentucky, Lexington, USA. The choice of accessions for this study was based on the available information on resistance to arthropod pests and/or reported chemical constituents of glandular trichomes.

Seeds of the accessions were acquired from the Genetic Resources unit of the United States Department of Agriculture (USDA) Plant Introduction Station at Geneva, New York, and from the Tomato Genetic Stock Center (TGSC) at Davis, California. From each accession, 25 seedlings were raised in 128-celled (34 cm^3 cell size) styrofoam Speedling^R flats containing Jiffy^R soil mix, and 8 of them randomly chosen and transplanted into pots for the study. Eight plants of the cultivated tomato cv. Solar Set were included as a susceptible control. The experimental plants were grown in 1500 cm^3 pots containing standard soil mixture. The pots were placed on benches in insect proof, water-cooled fiber glass greenhouses. Supplemental lighting was provided using 40-watt cool white fluorescent and 300-watt incandescent light bulbs to obtain a 16:8 (L:D)h photoperiod and 120-126 microeinsteins $m^{-2}s^{-1}$ (Li-cor Quantum Meter, Model LI-185A, Lambda Inst. Corp., Lincoln, Neb.) light intensity. The lights were suspended above the plants and were raised as the plants grew. Plants were watered as necessary. Temperature in the greenhouses ranged from 17-26 ^{0}C and the air remained humid. The plants were pruned to one stem. The plants were evaluated for whitefly resistance in a choice bioassay, where the plants were mass infested with silverleaf whiteflies (SLWF) from a colony maintained in the laboratory on tomato cv. Florida

Lanai (see Chapter 2 for details). At 65 days after transplanting into the 1500 cm^3 pots, leaf samples were taken from each plant for the determination of trichome density and for the composition of glandular trichome secretions. Thin Layer Chromatography (TLC) and Gas chromatography (GC) analyses were performed on the trichome secretions extracted from the samples. Detailed procedures for determining trichome density and exudate composition are given below.

F_1 Seeds and Plants

On the basis of the chemical analyses and high rating for resistance to the silverleaf whitefly (SLWF)(see Chapter 2), specific plants from resistant accessions were selected and used in 14 crosses to produce interspecific hybrids between wild species. Ten crosses were also made between the selected resistant wild germplasm and the cultivated tomato. The crosses were made in the greenhouse during April/May 1995. The parents were grown from cuttings, whereas the interspecific F_1 hybrids and the cultivated tomato parents were grown from seed. All the materials were grown under identical conditions during evaluation for resistance and the type of chemicals exuded by the glandular trichomes. Effort was made to synchronize the developmental state of the clonal parental plants and the plants raised from seed. The plants were grown in 1500 cm^3 pots in greenhouse conditions described under parental materials above. This experiment was conducted in fall 1995 season. Eight plants of each of the hybrids and the parents were evaluated. Standard soil mixture was used in the pots, and plants were watered as necessary. The plants were pruned to one stem per plant.

Approximately 21-28 days after transplanting, the plants were mass infested with SLWF from a laboratory colony maintained on tomato cv. Lanai; and, subsequently, the plants were rated for whitefly resistance (see Chapter 4 for rating technique).

Trichome Density Determination

The types and density of trichomes present on the leaves were determined for both the parental and hybrid plants, 65 days after transplanting into the pots. Density of type I, IV, V, and VI trichomes was determined from the average of counts made in four microscope fields on each surface of the pair of lateral leaflets proximal to the terminal leaflet of the leaf at the fifth node, counting from the apex of the plant. A dissecting microscope (Wild-Heerbrugg, Heerbrugg, Switzerland) set at 50X was used. Trichomes were counted on the abaxial surface of one leaflet and on the adaxial surface of the other. The fields of view, located in the interior middle section of the leaflet and with equal number on each side of the midvein, measured approximately 3.0 mm^2 each. The area of the leaflets used for these determinations was measured using a portable electronic area meter (LI-COR model LI-3000, Lambda Instruments Corporation, Lincoln, Neb.).

Sampling and Extraction of Trichome Exudates

Leaflet samples were taken from the plants for the extraction of glandular trichome exudates 70 days after transplanting. During the evaluation of the parental accessions two samples of leaflet washes were made, one with methylene chloride and another with hexane as the solvent. For the F_1 evaluation, however, only hexane leaflet wash samples were obtained.

Hexane leaflet washes

Samples for leaflet washes consisted of 5 to 8 leaflets taken from the leaf at node number 5 (counting from the first unfolded leaflet at the apex). A pair of steel forceps was used to sever the leaflets from the plant and to transfer them directly into a 125 ml erlenmeyer flask. The flask was closed with aluminium foil and transported to the laboratory where 10 ml hexane was added to each sample. The flasks were agitated for 30 min under a fume-hood on a rotary shaker operated at 1 cycle per second. The leaflet wash was then decanted and filtered into a 16 X 150 mm glass culture tube (Fisher Scientific, Pittsburgh, PA) using Whatman grade '1PS' phase separating filter paper (Whatman International Ltd., Maidstone, England) mounted in a glass funnel. Prior to being used, filter papers were rinsed with hexane to remove silicates. The tubes and polypropylene stoppers were weighed before collecting the filtrate. After decanting the leaflet washes, the leaflets and the sides of the flask were rinsed twice using 3 ml of hexane on each occasion, and the rinsates similarly filtered into the tube containing the washes. The hexane was evaporated by blowing a regulated (0.7 psi) stream of ultra-high-purity N_2 on the surface of the liquid until dry. After capping the tube, it was re-weighed. The amber-colored dried leaflet washes were stored in a freezer at -20 ^{0}C until the time of analysis.

The leaflets from the extraction were flattened, placed in polythene bags and stored in a refrigerator to await leaf area measurement, which was done not later than 48 h after the extraction. Leaf area was measured by use of a portable area meter. The leaf areas were used later to calculate the concentration of exudates and their different constituents.

Methylene chloride leafwashes

The leaflets were sampled as described for hexane washes. The leaflets were steeped in 10 ml methylene chloride under a fume hood. After the flask and its contents were swirled for 15 seconds, the leaflets were removed using a pair of steel forceps, and the leaflet wash was decanted into a 20 ml liquid scintillation vial that had been weighed previously. The flask was then rinsed with 3 ml methylene chloride, and the rinsate was added to the scintillation vial. To remove free sugars from the leaflet washes, 7 ml of distilled water was pippeted into the scintillation vial containing the leaflet wash, and after capping, the vial and its contents were vortexed to a milky appearance. After allowing time for the two solvents to clearly separate, the top aqueous phase was removed by aspiration with a vacuum pump. The methylene chloride was then evaporated by standing the uncapped vial and its contents in a fume hood for 1-2 days. The vial and its contents were then re-weighed.

Determination of the Chemical Composition of Glandular Trichome Exudates

The concentrated extracts were redissolved at the rate of 10 mg extract in 1 ml hexane or methylene chloride, depending on the solvent that had been used in the extraction of the particular sample. Sub-samples were obtaine from this stock solution for TLC and GC chemical profiles and analysisis of acylsugar content and composition.

Thin-layer chromatography (TLC) profiles

Both normal and reverse-phase TLC were used to analyze the composition of the leaflet washes from the parental accessions. However, because normal-phase TLC for the parental accessions did not provide additional data, only reverse-phase TLC was conducted for the extracts from the F_1 hybrid plants. Normal-phase and reverse-phase TLC were conducted on silica gel plates and reverse phase (C_8) plates (Whatman Inc., Clifton, NJ) respectively. Before use, the reverse phase plates were washed through full migration with a 90:10 ratio of methanol:water ($MeOH:H_2O$), air dried under a hood to remove the solvent, and then heated in a 75-80 ^{0}C oven for at least one hour. Normal phase plates were similarly prepared except that they were washed with a 90:10 ratio of hexane:methyl-tert-butyl ether.

From the leaflet wash stock solution described above, five microliters containing 50 ug of the crude extract were spotted on the TLC plate. Standards were also spotted on each plate. For each parental genotype, a normal-phase and a reverse-phase TLC plate was prepared and eluted. Reverse- phase plates were eluted with

80:20 ($MeOH:H_2O$) and normal- phase plates were eluted with hexane:methyl-tert-butyl-ether. To visualize the constituents in the sample, the normal-phase plates were stained with 5-6 ml of vanillin (4-hydroxy-3-methoxybenzaldehyde) (Fisher Scientific), and the reverse-phase plates were stained with orcinol-ferric chloride (Bial's Reagent, Sigma). The plates were then heated in an 110-115 ^{0}C oven until maximum color was obtained in 5-10 min. Orcinol is used to visualize sugars whereas vanillin is less specific, but differentiates among ketones and hydrocarbon sesquiterpenes and their acids (J. C. Snyder, pers. comm.). On reverse-phase plates, orcinol stains sugar esters a brown color. Vanillin stains methyl ketones blue-green, sesquiterpene hydrocarbons dark blue, glucose and sucrose acyl esters and sesquiterpene acids brown. On each plate, standard lanes were spotted with leafwashes from L. pennellii LA 716 for acylsugars, L. hirsutum f. glabratum PI 134417 for methyl ketones, and L. hirsutum f. typicum PI 251303 for for sesquiterpene hydrocarbons and their acids. The accessions were compared with respect to the type of spots and their Rf's, using color intensity as an indicator of relative abundance of the component.

Gas chromatography (GC) profiles

Gas chromatography (GC) was used to evaluate leaflet washes from L. hirsutum f. glabratum, L. hirsutum f. typicum and L. peruvianum genotypes. It was used also in evaluating F_1 hybrids where neither of the parents used in the crosses was a L. pennellii. Samples were taken from the stock solution of leaflet washes prepared as described earlier. The injected sample was 0.2 ul. The equipment used was a

Hewlett Packard 5890A (HP 5890) gas chromatograph equipped with a flame ionization detector (FID), a 10 m x 0.53 mm RSL-150 column (J & W Scientific, Folsom, California), and a HP 3392A Integrator interface. The flow rate of the carrier gas He, was 1.8 ml/min with a 90 kPa head pressure. The temperatures used in the system were: injector, 240 °C; detector, 300 °C; oven program, 130 °C for one minute, then increased at 6 °C/min to 170 °C, then increasing at 10 °C/min to 250 °C then held for 20 min for a total run time of 35.66 min.

Leaflet washes of L. hirsutum f. glabratum PI 134417 and L. hirsutum f. typicum PI 251303 were used as standards for methyl ketones and sesquiterpene hydrocarbons (and their acids), respectively. Chromatograms for different taxa were visually compared with respect to the retention times of the major components. Taxa were compared on the basis of the pattern of peaks that were characteristic to a particular taxon. The concentration (ug/cm^2) of the major component(s), characteristic of plants within a form of a species were calculated based on the integrator units and leaf area. The effective total integrator units representing the detected volatiles was the balance after deducting the solvent peak. Individual components were a proportion of this effective integrator area. Accessions within a species or a form of a species were compared with respect to 1)differences in major components and 2)similarities and concentration of the major components that were held in common. Plants within an accession were compared with respect to the similarity in major components and their concentration. The probable identity of components at the peaks unique to the resistant plants was assigned based on the known profiles of the standards used for comparison.

Determination of the Composition and Concentration of Sugars in F_1 and Parental Genotypes

To determine the composition and quantity of sugar in the sample, the enzymatic (Trinder) colorimetric method (Sigma Chemical Co., 1995) for glucose was adopted and applied after base hydrolysis of acylglucoses and acylsucroses. The samples for the analyses were obtained from the stock solution prepared by redissolving the dried crude extract at 10 mg per 1 ml hexane.

For analysis, 50 ul and 200 ul of the stock solution (10 mg/ml) of crude extract was aliquoted into glass tubes, and dried by evaporating in a stream of ultra-high-purity N_2 to obtain 0.5 mg and 2 mg crude samples for glucose and sucrose determinations, respectively. For glucose determination, the 0.5 mg sample was dissolved in 166 ml MeOH and hydrolyzed in 33 ul of 0.04N sodium hydroxide (NaOH) at 85 ^{0}C for 5 min. The weak base hydrolyzes the sugar esters to liberate free sugars. Similar preparation was used for system check containing 0.5 mg extract of LA 716, a standard containing 0.4 mg glucose, and a blank tube containing the base and 166 ul MeOH. The samples were prepared in duplicate. After hydrolysis, 400 ul acetate buffer (10 mM sodium acetate, 150 mM sodium chloride, adjusted to pH 4.5 with acetic acid) was added to each tube. A set of tubes for the glucose oxidation reaction (Trinder) were prepared. The reaction tubes, each containing 1 ml Trinder reagent, were arranged in a 37 ^{0}C water-bath. To these tubes, the following quantities were aliquoted: 300 ul of the hydrolyzed sample, 300 ul of the hydrolyzed control (LA 716), 30 ul of the glucose standard, and 30 ul from the blank. By weight, each of the hydrolyzed leaf extracts contained 250.42 ug of extract. The standard tube contained 18.96 ug of glucose. The oxidation reaction (glucose is oxidized to gluconic acid) was allowed to proceed for 10 min, at which time 0.7 ml deionized water was added to the sample tubes, while 1 ml was added to each of the standard and blank tubes, bringing final volumes in all tubes to 2.0 ml. Light absorbance of the samples was measured against deionized water on a computerized spectrophotometer (Shimadzu UV2101 PC) at 505 nm.

For sucrose determination, the 2 mg sample was dissolved in 664 ul MEOH and hydrolyzed in 132 ul 0.25N NaOH at 90 °C for 4.5 min. The strong base hydrolyzes all the acylsugars to liberate sucrose and glucose and degrades the free glucose but not the sucrose. The samples were then neutralized with 40 ul 0.85N acetic acid and 800 ul of the acetate buffer was added. A standard tube containing 400 ug sucrose was prepared. The blank and the system check were similar to those described for glucose determination, but were neutralized with 0.85N acetic acid and only 200 ul acetate buffer was added. For inversion, three aliquots of 400 ul each were taken from each hydrolyzed sample of the test extracts. To two of these, 200 ul of acid invertase (1 mg/10 ml acetate buffer) (Grade VII, Sigma, St Louis) was added, whereas only 200 ul of buffer was added to the third sample. To the 443 ul in the standard sample, 200 ul acid invertase was added, whereas 200 ul of buffer was pipetted into the blank tube. After vortexing, all the tubes were incubated in a 37 °C water bath for 30 min. The enzyme converts the non-reducing sugar, sucrose, to the reducing (inverted) sugars glucose and fructose for the colorimetric assessment with

Trinder reagent. The samples were then removed and cooled in ice water. Another set of tubes was prepared each with 1 ml Trinder reagent and placed in a 37 ^{0}C water bath. When the inverted samples were cooled, 300 ul was aliquoted from each into the tubes containing Trinder reagent. From the standard and the blank, 30 ul were aliquoted for the same reaction. By weight, the aliquots for the oxidation reaction contained respectively, 244.48 ug of the crude extract and 18.66 ug sucrose for the standard. After 5 min, 0.7 ml deionized water was added to each crude extract sample oxidation tube, and 1 ml of the water was added to the standard and blank tubes bringing the final volume to 2.0 ml for all tubes. Light absorbances of the samples were measured as previously described.

The mean absorbance for glucose was corrected for the blank, and glucose concentration in the sample was calculated relative to the glucose standard. For sucrose, because the base did not completely destroy all the glucose, the absorbance for the estimation of sucrose was corrected for the absorbance obtained from the hydrolyzed but non-inverted sample. The quantity of sucrose was derived from the resulting net absorbance compared to the standard. The leaf area associated with each extract sample was used to estimate the concentration of the sugars in ug/cm^2 of leaf area for each genotype.

To compare genotypes, the concentration (ug/cm^2) of glucose, sucrose, and the total amount of sugar were transformed ($\log_{10}$+1) prior to the analysis of variance (ANOVA) using PROC GLM statement of SAS and means separation by the Duncan Multiple Range Test. Spearman correlations between the concentrations of glucose, sucrose, and total sugars, and 1) trichome density and 2) densities of whitefly lifestages were calculated using the PROC CORR of SAS (SAS Institute Inc., 1988).

Results

Thin-Layer Chromatograms of Leafwashes from Parental Accessions in Primary Screening

The chemical profiles revealed by TLC differed among species, among forms within species, among accessions, and in some instances, among plants of the same accession. Reverse-phase TLC using orcinol stain showed dense brown color bands of sugar esters in all Lycopersicon pennellii accessions except LA 2963, which had less dense bands (data not shown). In L. pennellii var. puberulum accessions, no bands were apparent (Fig. 3-1). Among accessions of L. pennellii that had bands, the number and polarity (Rf) of the bands differed among accessions (data not shown). Within the accession LA 1340 of L. pennellii, plants differed in the number and Rf of bands (Fig. 3-1)

Chemical profile differences among L. hirsutum accessions were discernible using vanillin stain either in normal-phase or reverse-phase TLC. Accessions of L. hirsutum. f. glabratum possessed blue-green bands characteristic of methyl ketones. The accession LA 1353 (lane I) was exceptional to other L. hirsutum f. typicum because it had blue-green bands with almost identical polarity as those found in some L. hirsutum f. glabratum (Fig. 3-2). L. hirsutum f. typicum accessions generally had non-polar dark blue bands of sesquiterpene hydrocarbons, and polar brown bands of

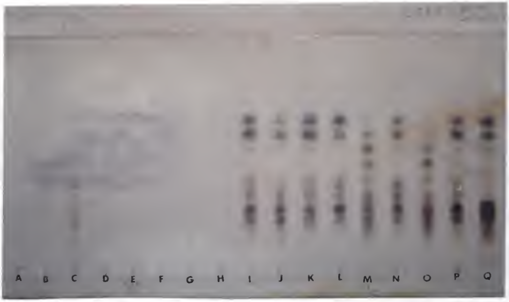


Figure 3-1. Thin layer chromatogram (C_8RP, orcinol stain) of exudates from glandular trichomes of different plants of L. pennellii var. puberulum accession LA 1911 (lane A-H), and L. pennellii accessions LA 1340 (lane I-P) and LA 716 (lane Q) extracted with hexane and visualized using orcinol stain.

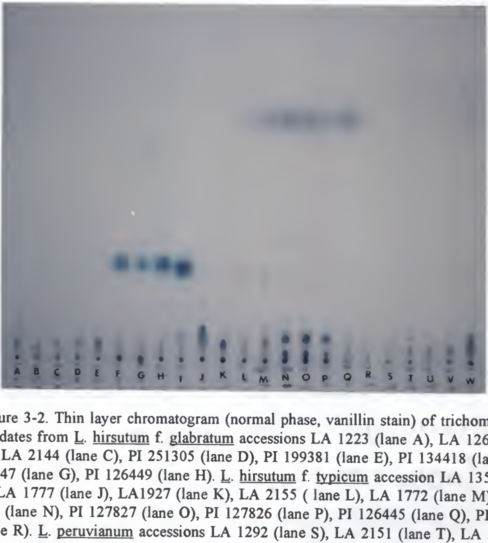


Figure 3-2. Thin layer chromatogram (normal phase, vanillin stain) of trichome exudates from L. hirsutum f. glabratum accessions LA 1223 (lane A), LA 1265 (lane B), LA 2144 (lane C), PI 251305 (lane D), PI 199381 (lane E), PI 134418 (lane F), PI 13447 (lane G), PI 126449 (lane H). L. hirsutum f. typicum accession LA 1353 (lane I), LA 1777 (lane J), LA1927 (lane K), LA 2155 (lane L), LA 1772 (lane M), LA 386 (lane N), PI 127827 (lane O), PI 127826 (lane P), PI 126445 (lane Q), PI 390513 (lane R). L. peruvianum accessions LA 1292 (lane S), LA 2151 (lane T), LA 364 (lane U), PI 127830 (lane V), and L. esculentum cv. Solar Set (lane W).

sesquiterpene acids. Additional polar blue-green bands were present in the profiles of accessions LA 386, LA 127826, and LA 127827 when viewed with vanillin stain in normal phase TLC (Fig. 3-2). Intra-accession variation for the number of chemical component bands was observed in the reverse phase profiles of LA 386, PI 127827, and PI 127826 (Fig. 3-3). No bands were observed in the profiles of plants of PI 199381, LA 2144, LA 1223, LA 1265, PI 390513, LA 2155 in normal-phase TLC (data not shown).

The TLC profiles of leafwashes obtained using either hexane or methylene chloride were also compared. For L. pennellii, the profiles obtained were similar; however, bands in the profiles of hexane leafwashes were slightly better defined (Fig. 3-4). There were also differences in the density of the bands, but there did not seem to be any pattern to these differences. Leafwashes of L. hirsutum f. typicum and L. hirsutum f. glabratum extracted with either solvent mainly gave similar chemical profiles (data not shown). Differences, however, occurred in the profiles of some plants of accession LA 386 and PI 127827.

Chemical profiles of L. peruvianum accessions and L. esculentum cv. Solar Set did not possess any bands under reverse phase TLC analysis (Fig. 3-1 and 3-2).

Gas Chromatograms of Leafwashes from Parental Accessions in Primary Screening

Chemical profiles of L. hirsutum f. glabratum, L. hirsutum f. typicum, L. peruvianum, and 'Solar Set' were determined by GC. The profiles differed between the species, forms of the species, among accessions, and within accessions. Despite the

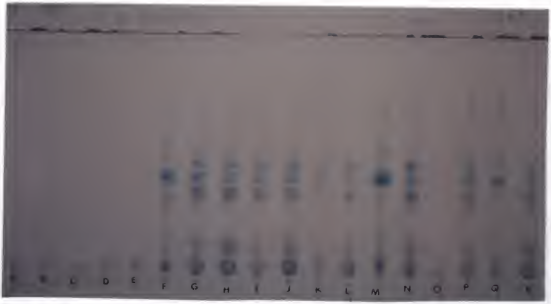


Figure 3-3. Thin layer chromatogram (C_8RP, vanillin stain) of exudates from glandular trichomes of L. hirsutum f. typicum accessions LA 386 (lanes F, G, H, I, and J), PI 127827 (lanes K, L, M, and N), PI 127826 (lanes O, P, Q, and R), L. peruvianum LA 2151 (lanes B, C, D, and E) and L. esculentum (lane A).

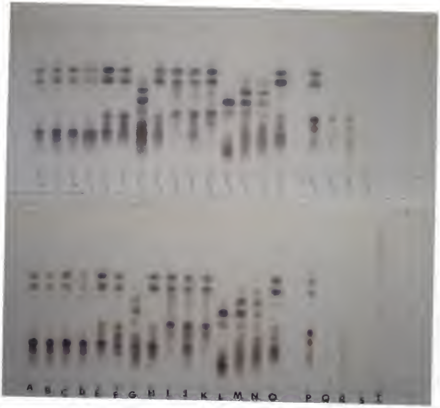


Figure 3-4. Thin layer chromatogram (C_8RP, orcinol stain) of exudates from glandular trichomes from L. pennellii accessions extracted using methylene chloride (top) or hexane (bottom), accession PI 246502 (lanes A and B), LA 716 (lanes C and D), LA 1340 (lanes E-H, and N and O), LA 1674 (lanes I-K), LA 2560 (lane L), LA 1302 (lane M), LA 2963 (lane P), LA 1911 (lanes Q and R), LA 1912 (lanes S and T).

variation, some components were frequently shared within an accession (Table 3-1). Accessions of L. hirsutum f. glabratum frequently shared two components with retention times of 2.23 and 4.43 (Fig. 3-5). The later eluting component was always in the larger proportion. Based on the profile of the known standard used, the components had elution times similar to 2-undecanone for the early eluter (2.24 min), and 2-tridecanone (4.43 min) for the later eluting component. In the accession LA 1265, no major components were found in the profiles of most of the plants; however, one plant had components with retention times of 5.55 and 12.63. There was greater diversity of components among L. hirsutum f. typicum, and none of the major components was found to be present in all the accessions analyzed. Plants of accession LA 1927 frequently had a component with retention time of 8.33 (Fig. 3-5). Plants of LA 386 frequently had common components with retention times of 4.37 and 4.58; however, one plant had a large amount of only one of them but not both. Within accessions PI 127826 and within PI 127827, the plants frequently had in common components with retention times of 4.36, 4.56, 6.66, and 7.50 (Fig. 3-5). Components frequently shared by plants of LA 1777 had retention times of 9.20, 9.30, 9.65, and 9.72 (Fig. 3-5). Plants of accession LA 1772 frequently shared components that had retention times of 4.36, 4.57, 9.20, and 9.87. The profiles of most plants of LA 1353 had in common components with retention times of 2.21, and 4.42 (Fig. 3-5). The early eluting component was found in larger proportions than the later one. Furthermore, the main components in this accession resembled those in L. hirsutum f. glabratum on TLC profiles and their retention times on the GC were

Table 3-1. Retention times, as determined by Gas Chromotography, and concentration of compounds frequently present at various levels in leaflet washes of L. hirsutum f. typicum and L. hirsutum f. glabratum accessions within which plants with varying levels of resistance to Bemisia argentifolii were found during a greenhouse choice bioassay. Fall 1994.

Taxon	Accession number	Resistance⁺ rating	Retention time	Concentration (µg/cm^2)	Component as per cent of total extract
L. hirsutum f. typicum					
	LA 386	3.3	4.38	15	8
			4.58	49	24
	PI 127826	3.4	4.36	9	4
			4.56	73	28
			6.66	30	9
			7.50	28	11
	PI 127827	3.9	4.36	9	5
			4.56	34	16
			6.66	21	10
			7.50	36	17
	LA 1772	4.0	4.36	7	3
			4.57	30	16
			9.20	5	3
			9.87	38	16
			10.11	54	21
			12.93	8	5
	LA 1927	4.5	8.33	116	43
	PI 126445	4.3	4.38	10	5
			4.57	19	10
	LA 1777	3.5	9.20	81	28
			9.65	67	23
			9.30	131	39
			9.72	92	27
	LA 1353	2.3	2.21	101	42
			4.42	6	2

Table 3-1 -- continued

Taxon	Accession number	Resistance[+] rating	Retention time	Concentration ($\mu g/cm^2$)	Component as per cent of total extract
L. hirsutum f. glabratum					
	PI 134417	3.4	2.24	4	1
			4.43	94	24
	PI 134418	3.6	2.24	2	1
			4.43	94	24
	PI 126449	3.5	2.24	3	1
			4.43	66	29
	LA 1265	4.3	5.55	17	10
			12.63	16	9

[+] mean for all tested plants of the accession - a maximum of 8 plants.

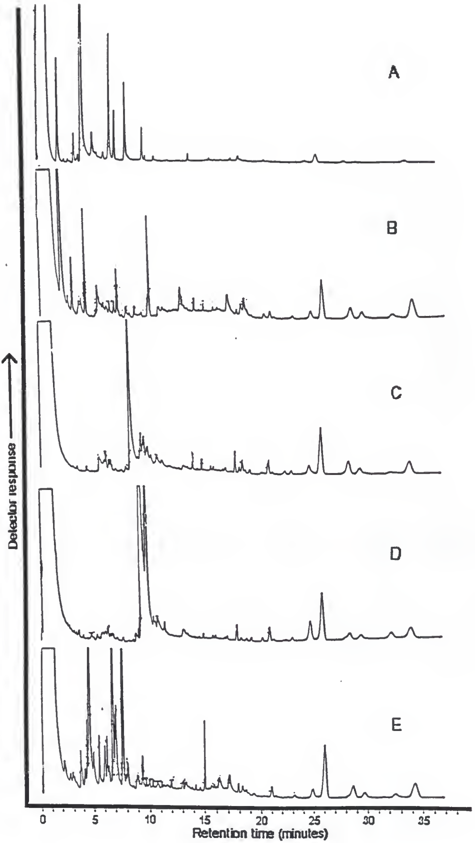
Key to rating: 1 = highly resistant; 2 = resistant; 3 = moderately resistant;
4 = susceptible; 5 = highly susceptible

slightly different, whereas their relative proportions were reversed. The chemical profiles of L. peruvianum and L. esculentum mostly had no major components.

Further Characterization of the Major Components in the Profiles of L. hirsutum f. typicum Accessions LA 386, PI 127826, and LA 1777

Thin layer chromatograms of extracts from LA 386, PI 127826, and LA 127827 revealed some polar blue-green bands that were uncharacteristic of L. hirsutum f. typicum accessions. Further investigations (J. C. Snyder pers. comm.) to better characterize the components were performed on extracts of LA 386, PI 127826, and LA 1777. These three accessions were chosen because they were later used in interspecific hybridization, and, therefore, further information on the basis for their resistance would be appropriate. For LA 386 and PI 127826, initial Gas Chromatography-Mass Spetrometry (GC-MS) analyses indicated that all the chemical components in the profiles of the two accessions were sesquiterpenoids. None of the components were base soluble whereas some can possibly silylate. Considering the GC-MS data, the TLC profiles, and the observations that they do not silylate and are not base soluble, one can conclude that the early eluting components are sesquiterpene hydrocarbons. Components eluting later are base insoluble and can probably silylate, and have molecular weights greater than those expected for a hydrocarbon. These preliminary results indicate that these components are sesquiterpenoid in nature, and that they likely contain oxygen, a pre-requisite for silylation (McGonigle, 1977). At this point, sesquiterpene lactones are one possible identity of these components. If this

Figure 3-5. Gas chromatograph profile of leaflet hexane extracts of exudates from glandular trichomes of one plant of the accession PI 134417 (A) of L. hirsutum f. glabratum, and LA 1353 (B), LA 1927(C), LA 1777(D), and PI 127826(E) of L. hirsutum f. typicum.


A
B
C
D
E
Detector response
0
5
10
15
20
25
30
35
Retention time (minutes)

proves to be the case, it will be the first report of its nature with regard to L. hirsutum. The investigation is continuing.

Preliminary results of GC-MS analysis of extracts of LA 1777 indicate that the major components are sesquiterpenoids, and that they are soluble in NaOH. This evidence indicates the components are probably sesquiterpene acids, the same acids identified by Coates et al. (1988).

Greenhouse Choice Bioassay for Resistance to SLWF and Chemical Profiles of Parental Accessions

All of the plants in the following groups were susceptible to SLWF: accessions LA 2963 of L. pennelli; accessions LA 1911 and LA 1912 of L. pennellii var. puberulum; accessions LA 1223, LA 2144, PI 251305 and PI 199381 of L. hirsutm f. glabratum; accessions LA 2155 and PI 390513 of L. hirsutum f. typicum; and accessions of L. peruvianum (see Chapter 2). The susceptible accessions had no major components in their TLC or/and GC profiles. Accessions in which resistant plants were observed and that had chemical components in their profiles are shown (Table 3-1). There were differences in the levels of resistance among accessions in which volatile components were revealed in their chemical profiles. Resistance levels were also variable within the accessions.

Thin-Layer Chromatograms of Leaflet washes from F_1 Interspecific Hybrids

Chemical profiles of the F_1 interspecific hybrids differed both among and within crosses as revealed by reverse-phase TLC. Sugar esters were visualized using orcinol stain. The differences in profiles were in the number and polarity of bands. The following are descriptions of the features of bands for each cross compared to the parents and the differences among plants of the same cross.

F_1 (LA 1353 X LA 1340): L. hirsutum f.typicum X L. pennellii

The profiles of some plants had fewer bands than the parents (Fig. 3-6) while others had about the same number or more. Where fewer bands were present, it was the more polar bands that were missing.

F_1 (PI 127826 X LA 1674): L. hirsutum f.typicum X L. pennellii

In general, the profiles of the plants in this cross had some bands of lower polarity than any found on the LA 1674 parent. One offspring (lane D) had no bands in common with the L. pennellii parent (Fig. 3-7).

F_1 (LA 386 X LA 1340): L. hirsutum f.typicum X L. pennellii

The profiles of some of the hybrid plants in this cross had more bands, especially less polar bands, whereas in other profiles the more polar bands found on the L. pennellii parent were missing (Fig. 3-7). The concentration in the sample was less in lanes I-L and N.

F_1 (PI 127826 X LA 1340): L. hirsutum f.typicum X L. pennellii

Hybrid plants in this cross had profiles ranging from those that resembled the L. pennellii parental profile, to some with some bands of lower polarity, and still others with more polar bands (Fig. 3-8). Plants whose profiles are in lane A-H were products of a cross involving the LA 1340 parent shown in lane T, whereas the hybrid plants in lanes I-P were derived from an LA 1340 plant (not shown in figure) lacking the more polar bands.

F_1 (LA 2560 X LA 1777): L. pennellii X L. hirsutum f. typicum

The hybrid plants in this cross had novel bands that were more polar than any found in the L. pennellii parent. There were also novel bands that were intermediate in polarity to those found in the profile of the parent (Fig. 3-9).

F_1 (LA 2560 X LA 1353): L. pennellii X L. hirsutum f. typicum

Three quarters of the plants had profiles similar to the parent LA 2560, whereas the remaining plants lacked the most polar band of the LA 2560 parent (Fig. 3-9).

F_1 (LA 1340 X LA 1777): L. pennellii X L. hirsutum f. typicum

The chemical profile of the hybrids in this cross contained bands of much lower polarity than those found on the L. pennelli parent. The number of bands, however, did not appear to differ from LA 1340 (Fig. 3-10).

F_1 (LA 1340 X PI 127826): L. pennellii X L. hirsutum f. typicum

Compared to the parent L. pennellii, the chemical profiles of the hybrids possessed a greater number of bands. The new bands were of lower polarity than any found on the parental profile (Fig. 3-10).

F_1 (LA 1340 X LA 386): L. pennellii X L. hirsutum f. typicum

In the profiles of the hybrids, the number of bands was generally greater. The novel bands were of lower polarity than those found on LA 1340 (Fig 3-10).

F_1 (LA 386 X LA 1674): L. hirsutum f. typicum X L. pennellii

More bands were observed in the hybrids, and the novel bands were of lower polarity than any found in the profile of LA 1674 (Fig 3-11). The profile of LA 386 also revealed sugar esters.

F_1 (LA 1353 X LA 1674): L. hirsutum f. typicum X L. pennellii

The hybrids possessed more acylsugar bands than the L. pennellii parent. The novel bands were of lower polarity (Fig 3-11).

F_1 (LA 2560 X PI 127826): L. pennellii X L. hirsutum f. typicum

The profiles in this cross were similar among hybrid plants. One of the bands found in the profile of LA 2560 was missing in the hybrids (Fig 3-12).

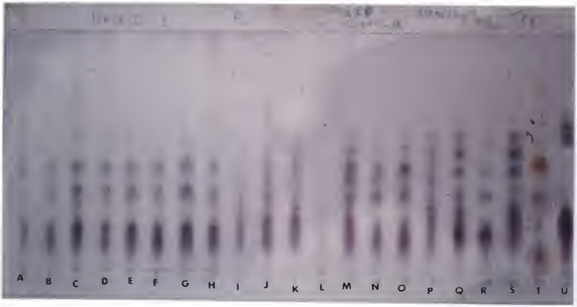


Figure 3-6. Thin layer chromatogram (C_8RP, orcinol stain) of exudates from glandular trichomes of interspecific hybrid F_1 (LA 1353 x LA 1340; lanes A-P), and parents LA 1340 (lane S), LA 1353 (lane R), and a physical mixture (1:1) of their exudates (lane Q).

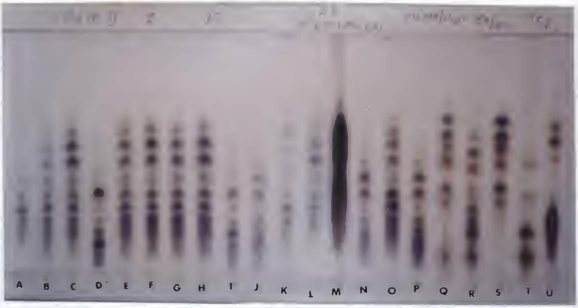


Figure 3-7. Thin layer chromatogram (C_8RP, orcinol stain) of exudates from glandular trichomes of interspecific hybrid plants (PI 127826 x LA 1674; lanes A-H), plants of F_1 (LA 386 x LA 1340; lanes I-P), physical exudate mixtures (1:1) of PI 127826/LA 1674 (lane Q), LA 386/LA 1340 (lane R). Parents LA 1674 (lane S) LA 386 (lane T), and LA 716 (lane U).

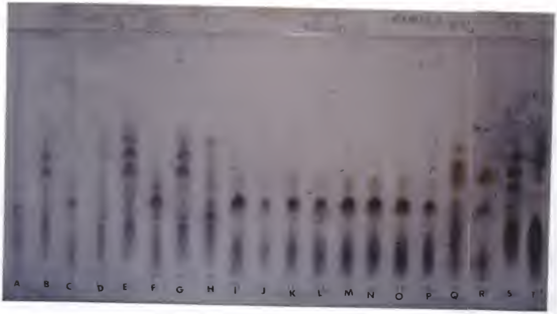


Figure 3-8. Thin layer chromatogram (C_8RP, orcinol stain) of exudates from glandular trichomes of interspecific hybrids F_1 (PI 127826 x LA 1340(a); lanes A-H), and F_1 (LA 127826 x LA 1340(b); lanes I-P), physical exudate mixtures (1:1) of PI 127826/LA 1340(a) (lane Q), and parents PI 127826 (lane R) and LA 1340(a) (lane S). LA 716 (lane T).

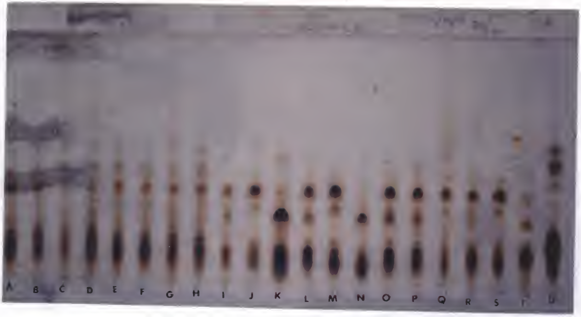


Figure 3-9. Thin layer chromatogram (C_8RP, orcinol stain) of exudates from glandular trichomes of interspecific hybrids F_1 (LA 2560 x LA 1777; lanes A-H), F_1 (LA 2560 x LA 1353; lanes I-P), physical mixtures (1:1) LA 2560/LA 1777 (lane Q) and LA 2560/LA 1353 (lane R). LA 2560 (lane S), LA 1353 (lane T), and LA 716 (lane U).

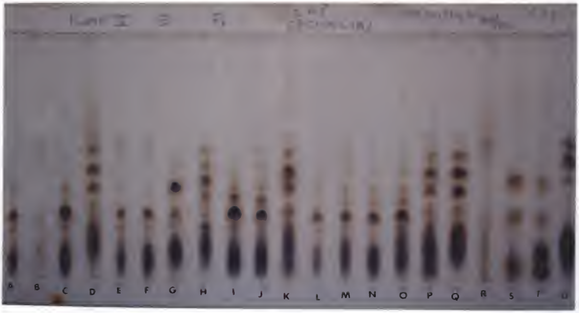


Figure 3-10. Thin layer chromatogram (C_8RP, orcinol stain) of exudates from glandular trichomes of interspecific hybrids F_1 (LA 1340 x LA 1777; lanes A-C), physical mixture (1:1) of LA 1340/LA 1777 (lane D), F_1 (LA 1340 x PI 127826; lanes E-J), physical mixture (1:1) of LA 1340/127826 (lane K), F_1 (LA 1340 x LA 386; lanes L-O), mixture (1:1) LA 1340/LA 386 (lane P), LA 1340 (lane Q), LA 1777 (lane R), PI 127826 (lane S), LA 386 (lane T), and LA 716 (lane U).

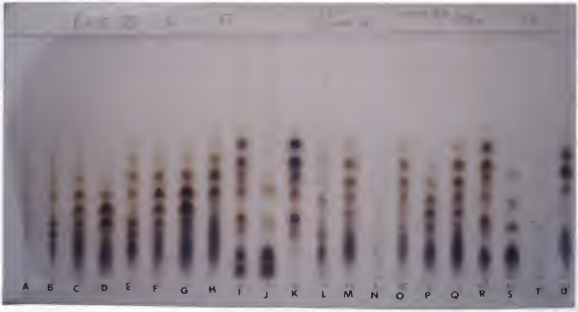


Figure 3-11. Thin layer chromatogram (C_8RP, orcinol stain) of exudates from glandular trichomes of interspecific hybrids F_1 (LA 386 x LA 1674; lanes A-H), LA 1674 (lane K), LA 386 (lane J), exudate mixture (1:1) LA 1674/LA 386 (lane I), F_1 (LA 1353 x LA 1674; lanes L-Q), physical exudate mixtures (1:1) of LA 1353/LA 1674 (lane R), LA 1353 (lane S), PI 126449 (lane T), and LA 716 (lane U).

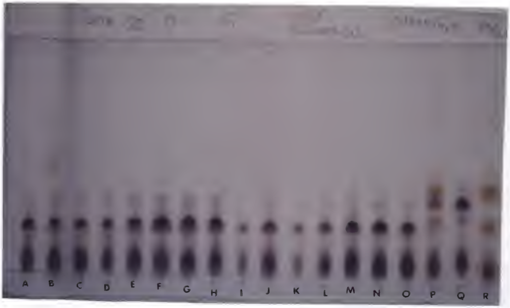


Figure 3-12. Thin layer chromatogram (C_8RP, orcinol stain) of exudates from glandular trichomes of interspecific hybrids F_1 (LA 2560 x PI 127826; lanes A-O), physical mixture (1:1) of exudates of the parents (lane P), LA 2560 (lane Q), and PI 127826 (lane R).

F_1 (LA 1674 X LA 1353): L. pennellii X L. hirsutum f. typicum

Of the two plants evaluated, one had a profile similar to that of LA 1674. The profile of the second plant had one novel band that was of lower polarity than any in the parent (data not shown).

F_1 (LA 1674 X LA 1777):L. pennellii X L. hirsutum f. typicum

The chemical profiles of half the hybrid plants were similar to that of LA 1674. The balance of the plants had fewer bands, and those bands were of lower polarity (data not shown).

Three breeding lines of L. esculentum were used in crosses with some of the resistant wild plants. There was variation among crosses and among plants of the same cross in the chemical profiles revealed by TLC. In the results below, the tomato lines are identified as: Fla 7324, Fla 7171, and Fla 952050. The TLC data for these crosses are not shown.

F_1 (Fla 7324 X LA 1340): L. esculentum X L. pennellii

Although the profiles of the hybrid plants resembled that of LA 1340, there were differences among the plants. One plant had no bands, one plant possessed one band that was of lower polarity than those in the parent. Other plants had some bands missing, especially parental bands of high polarity. The bands in hybrid profiles were generally faint.

F_1 (Fla 7171 X LA 1340): L. esculentum X L. pennellii

All the plants evaluated in this cross possessed bands. There was variation in the profiles among plants. One hybrid plant possessed a novel band that was of higher polarity than any found on LA 1340.

F_1 (Fla 7324 X LA 2560): L. esculentum X L. pennellii

Most of the hybrid plants in this cross had no discernible bands whereas the rest possessed fewer bands relative to the sugar ester profile of LA 2560.

F_1 (Fla 952050 X LA 2560): L. esculentum X L. pennellii

Some hybrid plants had no bands wheras others possessed fewer bands than found in LA 2560.

F_1 (Fla 7324 X LA 1674): L. esculentum X L. pennellii

Some plants had no bands. One plant exceeded the number of bands found in LA 1674. Generally, in the plants that possessed bands, the bands were of lower polarity than those found in LA 1674.

F_1 (Fla 7324 X other accessions)

The hybrid plants of the crosses; (Fla 7324 X LA 1777), (Fla 7234 X LA 386), and (Fla 7324 X PI 127826) possessed no bands in their TLC profiles.

The visualization of methyl ketones, sesquiterpene hydrocarbons, and sesquiterpene acids on reverse-phase TLC plates spotted with the leafwashes from the hybrids was undertaken by staining with vanillin. In general, the sugar ester bands present in the chemical profiles appeared to overlay, and, therefore, affect the visual detection of any other bands in the profiles. Such overlaying made it difficult to clearly demonstrate that in the chemical profiles of the hybrids, there was concurrent presence of the sugar esters and chemical components unique to the L. hirsutum parents.

With close inspection of the TLC chemical profiles, there were relatively stronger indications of the presence of bands other than sugar ester bands in some plants of the following hybrids:

F_1 (LA 1353 X LA 1340)-Fig. 3-13; L. hirsutum f. typicum X L. pennellii
F_1 (PI 127826 X LA 1674)- Fig 3-14; L. hirsutum f. typicum X L. pennellii
F_1 (PI 127826 X LA 1340) - Fig 3-15; L. hirsutum f. typicum X L. pennellii
F_1 (LA 2560 X LA 1777) - Fig. 3-16; L. pennelli X L. hirsutum f. typicum
F_1 (LA 2560 X PI 127826) - Fig. 3-17; L. pennelli X L. hirsutum f. typicum

Because of the inadequacy of the this technique to visualize sesquiterpene hydrocarbons in the presence of sugar esters, it is highly suspected that some plants in the hybrid plants from the other crosses may also contain these compounds in their trichome exudates.

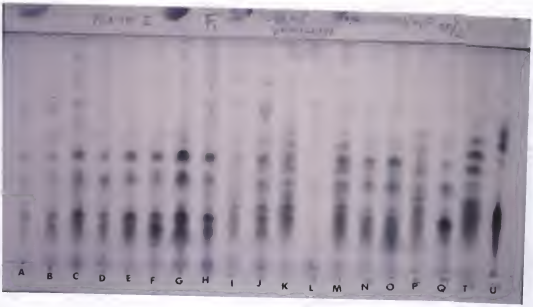


Figure 3-13. Thin layer chromatogram (C_8RP, vanillin stain) of exudates fromglandular trichomes of interspecific hybrids F_1 (LA 1353 x LA 1340; lanes A-P), LA 1353 (lane Q), LA 1340 (lane T), and LA 716 (lane U).

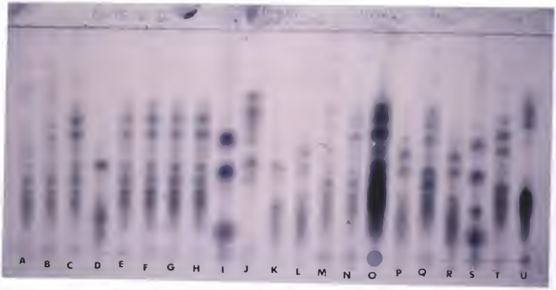


Figure 3-14. Thin layer chromatogram (C_8RP, vanillin stain) of exudates from glandular trichomes of interspecific hybrids F_1 (PI 127826 x LA 1674; lanes A-H), PI 127826 (lane I), LA 1674 (lane J), F_1 (LA 386 x LA 1340; lanes K-R), LA 386 (lane S), LA 1340 (lane T), and LA 716 (lane U).

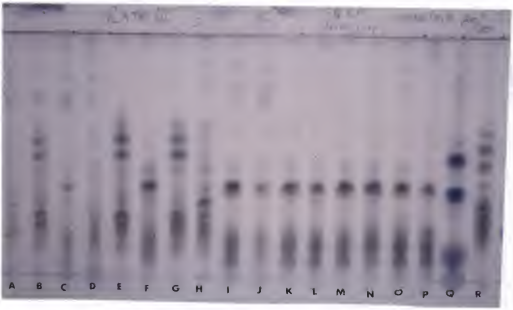


Figure 3-15. Thin layer chromatogram (C_8RP, vanillin stain) of exudates from glandular trichomes of interspecific hybrids F_1 (PI 127826 x LA 1340; lanes A-P), PI 127826 (lane Q), and LA 1340 (lane R).

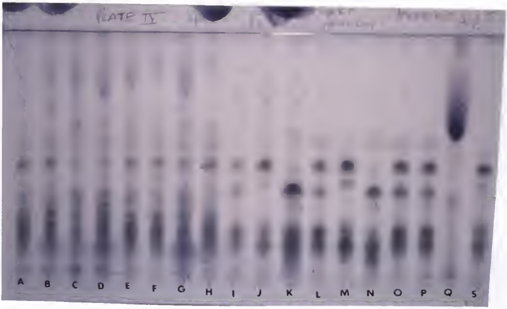


Figure 3-16. Thin layer chromatogram (C_8RP, vanillin stain) of exudates from glandular trichomes of interspecific hybrids F_1 (LA 2560 x LA 1777; lanes A-H), LA 1777 (lane Q), and LA 2560 (lane S).

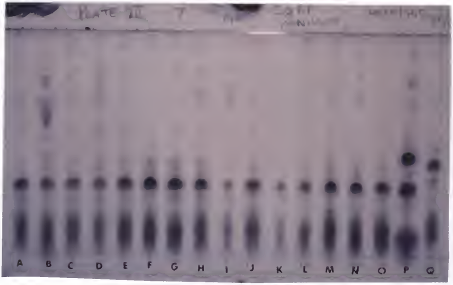


Figure 3-17. Thin layer chromatogram (C_8RP, vanillin stain) of exudates from glandular trichomes of interspecific hybrids F_1 (LA 2560 x PI 127826; lanes A-O), PI 127826 (lane P), and LA 2560 (lane Q).

Gas Chromatograms of F_1 Interspecific Hybrids

Gas chromatography was performed for L. esculentum X L. hirsutum hybrids, but not for hybrids involving L. pennellii because acylsugars in extracts from the latter hybrids would interfere with the functioning of the GC column.

F_1 (Fla 7324 X LA 386): L. esculentum X L. hirsutum f. typicum

Four hybrid plants were evaluated from this cross. three had no major peaks, but one of the plants had peaks that exceeded 10% of the net integrator area at 4.42 min. and 14.50 min. The parent LA 386 had a peak at 4.42 min. The other peak was not characteristic of the profile of the parents.

F_1 (Fla 7324 X PI 126449): L. esculentum X L. hirsutum f. glabratum

One plant from this hybrid had a major peak (32%) at 14.51 min; otherwise, the other seven evaluated plants had no major peaks.

F_1 (Fla 7324 X LA 1777): L. esculentum X L. hirsutum f. typicum

There was variability in the GC profiles among hybrid plants from this cross. One of the four plants had no major peaks. Three of the plants had common peaks at 3.52 min, and 3.91 min; and one of these plants had another peak at 5.26 min, whereas another plant had a peak at 14.48 min.

F_1 (Fla 7324 X PI 127826): L. esculentum X L. hirsutum f. typicum

Seven of the eight plants evaluated from this cross had no major peaks whereas one plant had a peak at 7.34 min.

Greenhouse Choice Bioassay for Resistance to the SLWF and Chemical Profiles of F_1 Interspecific Hybrids

The hybrids from crosses between L. pennellii and L. hirsutum f. typicum were either resistant or highly resistant to the SLWF (Table 3-2) and had minimal within hybrid variation (data not presented). Variation for resistance within hybrids from L. esculentum X L. pennellii crosses was apparent with some plants being resistant whereas others were highly susceptible. Densities of all immature lifestages of the SLWF were negatively and significantly correlated with the concentration of glucoses, sucroses, and total sugars (Table 3-3). The density of dead whiteflies was positively and significantly correlated to the sugars, but there was no correlation between the density of live adults and the concentration of sugars. Hybrids of crosses between the tomato and L. hirsutum were on average susceptible, with only one moderately resistant plant in each of the crosses with LA 386, PI 127826, and PI 126449.

Composition and Concentration of Sugar in F_1 Interspecific Hybrids, and the Relationship with Trichome Densities

Differences among genotypes assayed for sugars were significant for crude leaf extract, glucoses, sucroses, and total sugars (Appendix B). The differences among the F_1s were not significant for crosses between L. pennellii and L. hirsutum f. typicum.

Table 3-2. The composition and concentration of sugars ($\mu g/cm^2$) and the Bemisia argentifolii resistance rating for parental genotypes and F_1 interspecific hybrids between the wild L. pennellii, L. hirsutum, and L. esculentum evaluated in a greenhouse multiple choice bioassay - Fall 1995.

Genotype	Taxon$^+$	Number of plants	Glucoses	Sucroses	Total sugars	Sugars as percent of total extract	Resistance* rating
F_1(LA 1340 X PI 127826)	pen x typ	6	30(3) c-f	132(18) a	162(20) a-d	28	1.5
F_1(LA 1340 X LA 386)	pen x typ	4	16(2) e-h	97(7) a	113(8) a-d	17	1.0
F_1(LA 1340 X LA1777)	pen x typ	3	22(3) c-g	45(15) a-c	66(28) c-e	6	2.0
F_1(LA 1674 X LA 1353)	pen x typ	3	93(36) bc	81(41) ab	174(6) a-c	21	1.0
F_1(LA 1674 X LA1777)	pen x typ	3	84(18) b-d	75(19) ab	159(37) a-d	23	1.3
F_1(LA 2560 X PI 127826)	pen x typ	16	21(1) d-g	103(3) a	124(4) a-d	26	1.1
F_1(LA 2560 X LA 1353)	pen x typ	8	51(6) c-e	87(6) ab	138(10) a-d	27	1.1
F_1(LA 2560 X LA 1777)	pen x typ	8	48(1) b-e	79(2) ab	126(3) a-d	22	1.0
F_1(PI 127826 X LA 1340)	typ x pen	15	42(3) c-f	96(6) ab	139(8) a-d	28	1.3
F_1(PI 127826 X LA 1674)	typ x pen	8	49(3) c-e	132(5) a	181(6) a-c	28	1.0
F_1(LA 1353 X LA 1340)	typ x pen	16	21(1) e-h	66(3) ab	87(4) b-e	18	1.4
F_1(LA 1353 X LA 1674)	typ x pen	7	43(4) c-f	53(7) a-c	96(12) b-e	17	1.0
F_1(LA 386 X LA 1340)	typ x pen	9	32(2) c-f	147(9) a	178(10) a-c	26	1.0
F_1(LA 386 X LA 1674)	typ x pen	8	33(2) c-f	122(13) ab	155(14) a-d	26	1.4
F_1(FLA 7324 X LA1340)	esc x pen	8	27(2) c-g	54(2) ab	81(5) a-d	25	2.5
F_1(FLA 7171 X LA 1340)	esc x pen	8	30(1) c-f	51(4) ab	81(6) a-d	19	2.1
F_1(FLA 7324 X LA 1674)	esc x pen	6	18(2) e-h	26(3) a-d	44(5) d-f	10	3.8
F_1(FLA 7324 X LA 2560)	esc x pen	8	6(1) g-j	7(2) c-e	12(2) g	4	3.9
F_1(FLA 952050 X LA 2560)	esc x pen	7	9(1) f-i	14(3) b-d	23(4) e-g	9	3.0

Table 3-2 -- continued

Genotype	Taxon[+]	Number of plants	Glucoses	Sucroses	Total sugars	Sugars as percent of total extract	Resistance* rating
F$_1$(FLA 7324 X LA 1777)	esc x typ	3	6(1) g-i	2(1) de	12(1) fg	<1	5.0
F$_1$(FLA 7324 X PI 127826)	esc x typ	8	<1 k	<1 e	<1 g	<1	4.8
F$_1$(FLA 7324 X LA 386)	esc x typ	3	3(1) i-k	3(2) de	6(1) g	1	4.3
F$_1$(FLA 7324 X PI 126449)	esc x glab	8	5(1) h-k	-	-	-	4.4
F$_1$(FLA 7171 X PI 126449)	esc x glab	2	5(0) g-j	0 e	5(0) g	<1	5.0
LA 1340	pen	8	160(-) ab	80(-) ab	240(-) ab	22	1.0
LA 1674	pen	8	277(-) a	18(-) a-d	295(-) a	24	1.1
LA 2560	pen	8	53(-) b-e	54(-) ab	107(-) a-d	11	1.1
PI 127826	typ	8	11(4) f-i	5(-) c-e	16(-) fg	5	2.4
LA 1353	typ	8	3(-) i-k	88(-) a	91(-) a-d	21	1.0
LA 386	typ	8	19(-) d-g	29(-) a-c	47(-) d-f	10	1.1
LA 1777	typ	8	19(-) d-g	23(-) a-c	42(-) d-f	7	1.4
PI 126449	glab	4	3(-) i-k	0 e	3(-) g	<1	3.5
FLA 7324	esc	8	<1 jk	<1 e	<1 g	<1	5.0
FLA 7171	esc	8	<1 jk	<1 e	<1 g	1	5.0
FLA 952050	esc	8	<1 jk	<1 e	<1 g	<1	5.0

Mean of at least 8 plants for the hybrids and 4 plants for the parental genotypes.

* Key to rating: 1 = highly resistant; 2 = resistant; 3 = moderately resistant; 4 = susceptible; 5 = highly susceptible.

Means(SEM) within a column followed by the same letter are not significantly different ($p<0.05$) by Duncan's Multiple Range Test.

Statistical analysis was performed on log. transformed data but means are original data.

[+] pen = L. pennellii; typ = L. trisutum f. typicum; glab = L. hirsutum f. glabratum; esc = L. esculentum.

Table 3-3. Correlation (r) between the concentration (μg/cm^2) of sugars , and the densities (cm^{-2}) of whitefly lifestages on the leaf surface of the 7th leaf node of F_1 interspecific hybrids between L. pennellii, L. hirsutum, and L. esculentum, and the parents, evaluated in greenhouse multiple choice bioassay for resistance to Bemisia argentifolii - Fall 1995.

Whitefly lifestage and leaf surface	glucoses	sucroses	Total sugars
Adaxial			
Eggs	-0.57***	-0.53***	-0.53***
Crawlers	-0.53***	-0.53***	-0.52***
2nd & 3rd Instars	-0.50***	-0.49***	-0.45***
Pupae	-0.49***	-0.41***	-0.39***
Exuviae	-0.41***	-0.35***	-0.32***
Live Adults	0.06ns	0.07ns	0.07ns
Dead Adults	0.53***	0.46***	0.47***
Abaxial			
Eggs	-0.60***	-0.62***	-0.60***
Crawlers	-0.57***	-0.53***	-0.52***
2nd & 3rd Instars	-0.59***	-0.57***	-0.56***
Pupae	-0.52***	-0.45***	-0.44***
Exuviae	-0.38***	-0.32***	-0.25**
Live Adults	-0.03ns	-0.01ns	-0.02ns
Dead Adults	0.47***	0.41***	0.42***

153 observations used in computing correlation coefficients.
ns, *, **, *** = nonsignificant(ns), or significant at 0.05(*), 0.01(**), and 0.001(***) level.

The hybrids between L. pennellii and the tomato, had generally less sugars except for crosses involving LA 1340, whose sugar levels were comparable to the former hybrids (Table 3-2). In the hybrids the sucroses were generally more abundant than glucoses, wheras in the parental L. pennellii the reverse was true for LA 1340 and LA 1674. However, LA 2560 had the two sugar types in almost equal proportions.

Correlation between type I and IV trichomes density and glucoses, sucroses, and total sugars was positive and significant, whereas type VI and V and the sugars were negatively but significantly correlated (Table 3-4).

Discussion

The TLC and GC profiles of the tested Lycopersicon germplasm revealed the differences of main chemical constituents among species, forms of species, accessions of a species, and plants within some accessions. L. pennellii var. puberulum and L. peruvianun differed from the other species because no bands or peaks were revealed in their chemical profiles. The other species differed in the chemical nature of the main constituents. The main constituents in L. pennelli were acylsugars, which were not detected in the L. pennellii var. puberulum. This is in agreement with Shapiro et al. (1994), who did not detect any acylsugars in the L. pennelli var. puberulum accessions that they evaluated. Main constituents in L. hirsutum f. glabratum were methyl ketones. L. hirsutum f. typicum had more variety of main constituents, with sesquiterpene hydrocarbons and their acids being the most frequent. Other major

Table 3-4. Correlation (r) between the concentration (μg/cm^2) of sugars secreted and the types and densities of trichomes on the surfaces of the 5th leaf node on plants of the parents and F_1 interspecific hybrids between L. pennellii, L. hirsutum, and L. esculentum evaluated in greenhouse multiple choice bioassay for resistance to Bemisia argentifolii - Fall 1995.

Trichome type and leaf surface	glucoses	sucroses	Total sugars
Adaxial			
type I	0.36***	0.39***	0.30***
type IV	0.46***	0.59***	0.49***
type V	-0.60***	-0.57***	-0.58***
type VI	-0.30***	-0.33***	-0.26***
Abaxial			
type I	0.33***	0.28***	0.18*
type IV	0.40***	0.55***	0.47***
type V	-0.59***	-0.59***	-0.60***
type VI	-0.20***	-0.33***	-0.29***

153 observations used in computing correlation coefficient value.

*, **, *** = significant at 0.05(*), 0.01(**), 0.001(***) level.

components included unconfirmed methyl ketone-like constituents in LA 1353 and some polar, silylating, not base hydrolyzable sesquiterpenoids in accessions LA 386, PI 127826, and PI 127827. Weston et al. (1989) reported the occurrence of sesquiterpenes and methyl ketones in leaflet washes of L. hirsutum f. typicum and L. hirsutm f. glabratum respectively. However, they also reported the presence of measurable concentrations of 2-tridecanone in the accession PI 127827, traces of methyl ketones in other accessions of L. hirsutum f. typicum, and traces of sesquiterpene hydrocarbons in some L. hirsutum f. glabratum accessions.

Differences in the main constituents among accessions of the species L. pennelli were revealed in the reverse phase TLC. The accession LA 2963 differed from the other accessions because it had very faint bands. Although the concentration of sugars was not quantified during the evaluation of parental genotypes, low concentration in this accession is suspected to be the explanation of this difference in band density. The other accessions of this species differed in the number and the general mean polarity of the bands. Shapiro et al. (1994) reported differences in the types and levels of acylsugars found in L. pennellii. In my evaluation, analysis of the type and concentration of acylsugars for accessions LA 1340, LA 1674 and LA 2560 revealed that these accessions did differ in the mean concentration of acylsugars. Those acylsugars consisted of acylglucoses and acylsucroses, and the proportions of these also differed among this limited set of accessions (Table 3-2).

Accessions of L. hirsutum f. glabratum differed in the absence of major constituents in some accessions, and differed in the identity of the major constituents as manifested in different TLC profile characteristics or retention times in GC profiles. However, where major components were present, these had the TLC color characteristics, and GC migration characteristic of methyl ketones of the known standard. Where components were found in common, concentration (ug/cm^2) differences among accessions were found (Table 3-1). Plants within an accession differed in color density of component bands in the TLC profiles, probably due to concentration differences, and the number of peaks in the GC profiles. There were also differences within accessions for the number of major component peaks in GC profiles.

One of the differences among accessions of L. hirsutum f. typicum was the absence of major components in the TLC and GC profiles of some accessions. The major components also differed among accessions as revealed by TLC and different retention times in the GC profiles. There were concentration differences among accessions for some major components that were common to the taxa (Table 3-1). LA 1353 differed from the other accessions by having major components that resembled methyl ketones on the TLC profiles, but deviated slightly in retention time from the suspected equivalent components in known standards. LA 1353 also possesed a relatively high concentration of acylsugar, mainly acylsucroses (Table 3-2). Some acylsugars were also detected in LA 1777 and LA 386. King et al. (1990) reported the occurrence of acylsucroses in the trichome exudates of the accession LA 1353, and King et al. (1993) reported the same in LA 1777. The main constituents in LA 1777 had TLC profile characteristics of sesquiterpene hydrocarbon acids. Coates et al.

(1988) identified alpha-santalenoic acid, beta-bergamotenoic acid, and alpha-bergamotenoic acids from hexane extracts of whole leaves of this accession. LA 386, PI 127826, and PI 127827 also differed from the other accessions because they had major components of a sesquiterpenoid nature, but were neither sequiterpene hydrocarbons nor their acids. These components were blue-green in color and polar on TLC profiles. On GC, they were relatively late eluting. Further investigation revealed that they were not base hydrolyzable and could probably silylate. Such components have not been reported previously for L. hirsutum, and the investigations on their identity is continuing.

The most conspicuous components in the TLC profiles of the interspecific F_1 hybrids were the acylsugars. Hybrid populations differed in homogeneity of the number and polarities of bands. Plants within a hybrid population differed in the number of bands and the polarity of the bands. Analysis of the composition and concentration of sugars indicated that hybrids of L. pennellii X L. hirsutum f. typicum generally possessed larger amounts of sugar than hybrids from crosses of either of these wild species and the cultivated tomato, L. esculentum (Table 3-2). The hybrids differed in the ratio of glucoses:sucroses, with ratios ranging from par to 1:5. Sucroses occurred in the larger concentration in more than 73% of the populations. Compared to other hybrid populations from crosses between L. esculentum and L. pennellii, the populations that had L. pennellii accession LA 1340 as a parent had a significantly larger amount of sugars than the rest.

Resistance to SLWF was associated with the major components in the profiles of Lycopersicon germplasm and their hybrids. Genotypes with no detectable components were susceptible. Some major components were found to be common among the more resistant accessions within a taxon; acylsugars, sesquiterpenoids, and methyl ketones in L. pennellii, L. hirsutum f. typicum, and L. hirsutum f. glabratum. This could be an indication that these components have a significant role in the expression of the resistance. However, the presence of major components did not guarantee resistance, and this differential is probably related to the concentration of the major components. Importance of the concentration of the components per unit area of leaf surface was best manifested in the reduced resistance of hybrids with less acylsugars. However, the aspect of concentration was not explicitly demonstrable for the methyl ketones, the sesquiterpenoids, and the yet unidentified major components found in some L. hirsutum f. typicum accessions. In comparison to the parental L. pennellii, there was an apparent decreasing trend in the concentrations of total sugars in the hybrids (Table 3-2) whereas high levels of resistance were maintained. It was not possible to discern the relative effects of the altered composition of sugars, and/or the presence of sesquiterpenoids and the unidentified components, on the expression of SLWF resistance. However, the maintenance of resistance in spite of decreased sugar could be an indicator of the significance of some, or all, of the changed chemical aspects. The full impact of these changes might become more evident in later generations of these hybrids, and especially when these materials are used as donors for resistance in hybrids with tomato.

CHAPTER 4

EVALUATION OF RESISTANCE TO THE SILVERLEAF WHITEFLY IN F_1 INTERSPECIFIC HYBRIDS BETWEEN LYCOPERSICON PENNELLII AND L. HIRSUTUM AND BETWEEN THE WILD SPECIES AND THE CULTIVATED TOMATO, L. ESCULENTUM

Introduction

The silverleaf whitefly (SLWF), Bemisia argentifolii Bellows & Perring, formerly biotype 'B' of the sweetpotato whitefly (SPWF) Bemisia tabaci (Gennadius), is a major pest of tomato, Lycopersicon esculentum Mill. in south Florida (Schuster et al., 1996). In 1992, infestation resulted in losses of 500 million dollars to US agriculture (Perring et al., 1993). This pest is difficult to control with insecticides because feeding and oviposition occur mainly on the lower surfaces of leaves (Sharaf, 1986), and because SLWF develops resistance to insecticides applied for its control (Dittrich and Ernest, 1990; Stansly et al., 1991). Having resistant cultivars to grow would be advantageous because they are compatible with other components of integrated pest management, conserve the environment, and slow the development of insecticide resistance through reduced applications. Currently, no commercial cultivars with resistance to the whitefly are available (Schuster et al., 1996) .

Tigchelaar (1986) noted the scarcity of commercial tomato cultivars that had been developed with specific resistances to insect pests. He submitted that this situation was partly a result of the difficulty of developing breeding procedures to use the variability that is available. High levels of resistance to the silverleaf whitefly were identified in several L. pennellii, and L. hirsutum accessions (see Chapter 2). Other workers (Berlinger et al., 1983; Shevach-Urkin, 1983), had previously attempted to use some of the same germplasm in breeding for resistance to the SLWF. No cultivar has been developed from these efforts, mainly because of the difficulty in transferring and retaining effective levels of the resistance while eliminating the wild characteristics of the donor parents (J. W. Scott pers. comm.). Barten et al. (1995) reported that the evaluation of the resistance in L. pennellii varied when tested by different methods and under different conditions. This might adversely affect the efficiency of identifying parents, and likely also the selection of progenies when introgressing the resistance into the cultivated tomato.

Accessions with different profiles for resistance chemicals were combined to generate an alternative resistance source to be used in breeding for resistance to the SLWF. It is anticipated that the resulting resistance might be more amenable to tomato breeding for resistance to the SLWF. The results of the evaluation of F_1 interspecific hybrids combining the different chemistries are presented here.

Materials and Methods

Selection of Parents and Cross Breeding

The process of identifying resistance sources for the SLWF started with the screening of 32 wild Lycopersicon accessions in a multiple choice bioasssay performed in a greenhouse at the GCREC, Bradenton, Florida during Fall 1994. The germplasm screened consisted of 8 accessions of *Lycopersicon pennellii* and 2 of the subspecies *L. pennellii* var. *puberulum*; 8 and 10 accessions of *L. hirsutum* f. *glabratum* and *L. hirsutum* f. *typicum*, respectively; and 4 accessions of *Lycopersicon peruvianum*. The accessions were included based on available information on resistance to arthropod pests and/or reported chemical constituents of glandular trichomes. The plants were evaluated for resistance during Fall 1994 after mass infestation with whiteflies from a laboratory colony of *B. argentifollii* that was maintained on the tomato cv. Florida Lanai. Glandular trichome exudates were extracted separately from leaflets of each plant, and their chemical profiles were analyzed by thin layer chromatography (TLC) and gas chromatography (GC). The plants that were found to be resistant were vegetatively propagated from cuttings. During Spring 1995 season, a field trial was conducted to verify the resistance that was found in 33 plants from 22 accessions. Plants to be used in interspecific hybridization were selected individually on the basis of their resistance and different TLC analysis.

The selected parental plants were either resistant or moderately resistant to the SLWF, and they represented a range of apparent chemical profiles revealed by the TLC analysis. There were three plants per accession selected from LA 1340, LA 1674, and LA 2560 of *L. pennellii*; LA 386, LA 1353, LA 1777 and PI 127826 of *L. hirsutum* f. *typicum*; and PI 126449 of *L. hirsutum* f. *glabratum*. Cuttings were made from the selected plants, rooted under intermittent mist, and then transplanted into greenhouse beds where the crossing was done. Breeding lines of the cultivated tomato used in the crosses were also grown in greenhouse beds. Interspecific crosses were made in April and May of 1995. Where a wild parent was the seed parent, pollinations were carried out without emasculation at anthesis, but cultivated tomato seed parents were emasculated manually one day prior to anthesis and pollinated on the following day.

Greenhouse Evaluation of F_1 Plants

F_1 hybrid seeds were extracted from mature ripe fruits. For each cross, and depending on the availability of seeds, 25 seedlings were raised in 128-celled (34 cm^3 cell size) styrofoam Speedling^R flats (Speedling Hort. Prod. Div., Sun City, Florida USA) containing Jiffy^R soil mix. At the third leaf stage of growth, eight were chosen randomly and transplanted into 1500 cm^3 pots containing a standard soil mixture. From each of the wild species parental plants, which had been maintained in the greenhouse, eight cuttings were made, rooted under intermittent mist, and then the rooted cuttings were transplanted into 1500 cm^3 pots. Eight plants from each of the tomato genotypes used as parents in hybridization were grown from seed. There were 24 F_1 hybrid populations, the 8 wild parental genotypes and 3 tomato breeder's lines (Fla 7324, Fla 7171, and Fla 952050) that had been used as parents.

The pots were placed on benches in water-cooled, insect proof, fiber glass greenhouses and were watered as necessary. Supplemental lighting was provided using 40-watt cool white fluorescent and 300-watt incandescent light bulbs to obtain a 16:8 (L:D)h photoperiod and 120-126 microeinsteins $m^{-2}s^{-1}$ (Li-cor Quantum meter, Model

LI-185A, Lambda Inst. Corp., Lincoln, Neb.) light intensity. The lights were suspended above the plants and were raised as the plants grew. Temperature in the greenhouses ranged from 17-26 ^{0}C and the air remained humid. The plants were pruned to one mainstem.

Approximately 21-28 days after transplanting, the plants were mass infested with SLWF from a colony maintained in the laboratory on tomato plants cv. Florida Lanai. The numbers of whitefly adults, eggs, 1st-4th instars, and empty pupal cases (emerged adults) were determined at 32 days after mass infestation of the greenhouse, when the plants had approximately ten true leaves. Four leaflets proximal to the terminal leaflet on the leaves at nodes 5 and 7 (counting from the first unfolded leaflet at the plant apex) were harvested using a pair of steel forceps to sever them from the plant and to transfer them directly into a 15 cm diameter plastic petri dish. The samples were taken to the laboratory and whitefly adults, eggs, crawlers, and nymphs were counted on each leaflet surface for all the eight leaflets using a dissecting microscope (Wild-Heerbrugg, Heerbrugg, Switzerland) set at 12X.

The density of type I, IV, V, and VI trichomes (sensu Luckwill, 1943) was determined from the average of counts made at four dissecting microscope fields on each surface of the pair of lateral leaflets proximal to the terminal leaflet on the leaf at the fifth node. One of the leaflets was used to determine trichome density on the adaxial leaflet surface and the remaining one was used for the abaxial surface. The dissecting microscope (Wild-Heerbrugg, Heerbrugg, Switzerland) set at 50X was used to count trichomes. The fields of view, located in the interior middle section of the leaflet and with equal number on each side of the midvein, measured approximately 3.0 mm^2 each. The area of the leaflets used for insect counts and trichome density was measured by use of a portable area meter (LI-COR model LI-3000, Lambda Instruments Corporation, Lincoln, Neb.).

Evaluation of F_1 Interspecific Hybrids for Field Resistance to the SLWF

Seed of F_1 interspecific hybrids was sown on 10 Sept. 1995 in 128-celled (34 cm^3 cell size) styrofoam Speedling^R flats (Speedling Hort. Prod. Div., Sun City, Florida USA) containing Jiffy^R soil mix. Cuttings from the parental plants were rooted as described above, whereas the cultivated tomato parents were raised from seed. Six seedlings per cross were set in the field at the Gulf Coast Research and Education Center, Bradenton, Florida on 11 October in the Fall 1995 season. The seedlings were set on 84 cm wide by 20 cm high beds of EauGallie fine sand covered with white polyethylene plastic mulch. The experiment was laid out in six randomized complete blocks with one plant for each genotype per block. Within the bed, plants were spaced 91 cm between one another. Blocks were arranged parallel to the irrigation ditches. Yellow squash (Cucurbita pepo L.), was planted at every other bed to serve as spreader rows to increase whitefly population pressure in the trial. Squash had been seeded on 22 Sept. and infested on 9 Oct. with SLWF from the laboratory colony. After the whitefly population built up on the squash plants, the plants were cut on 8 November. The test plants were staked and tied individually and standard growing practices were applied (Howe et al., 1995). Bacillus thuringiensis was applied to control lepidopteran insect pests but no other insecticides were used. There were 17 hybrids, and the parental genotypes consisting of eight wild Lycopersicon accessions, and the three cultivated L. esculentum lines, Fla 7324, Fla 7171 and Fla 952050.

The population density of whitefly adults, eggs, 1st-4th instars, and empty pupal cases (emerged adults) was determined on 10 Dec., 60 days after the seedlings were set in the field. Four leaflets proximal to the terminal leaflet from the leaf at node number seven (counting from the plant apex) of each of two plant shoots per plant were severed. The samples were taken to the laboratory and whitefly adults, eggs, crawlers, and nymphs were counted on each leaflet surface for all the eight leaflets. A dissecting microscope set at 12X was used for the counts. The pair of lateral leaflets proximal to the terminal one on the leaf at the fifth node was also sampled for trichome density determination. The density of type I, IV, V, and VI trichomes was determined using a procedure identical to the one described for the greenhouse experiment.

To compare genotypes, the whitefly densities and trichome densities were transformed (square root of $(X + 0.5)$) prior to analysis of variance (ANOVA) using PROC GLM statement (SAS Institute Inc., 1988) and means separation was performed by the Duncan's Multiple Range Test. Spearman correlations between trichome densities and insect densities were calculated using the PROC CORR.

Results

Greenhouse Evaluation

Genotypes differed significantly for the density of eggs, crawlers, 2nd and 3rd instars, pupae, and exuviae, on both the adaxial and abaxial surfaces of the leaves (Appendix C-1, Tables 4-1 and 4-2). The genotypes also differed significantly for dead adults, but not for live adults on either surface of the leaves.

For the immature whitefly stages on the adaxial surface (Table 4-1), the cultivated tomato genotypes had the highest density for most stages. The hybrids and the wild Lycopersicon parents were rarely distinguishable in the density of immatures on the adaxial surface. On the abaxial surface, however, some hybrids between wild and the cultivated tomato were grouped together with the cultivated tomato for the highest density of immatures (Table 4-2). The general trend was for the wild parents and the hybrids between L. pennelii and L. hirsutum f. typicum to have the least density, followed by hybrids between the cultivated tomato and the wild species, and then followed by the tomato genotypes. LA 1340 had the highest density of dead adults on the abaxial surface, whereas all the other genotypes were not significantly different from each other.

The F_1s between L. hirsutum f. typicum X L. pennellii did not differ significantly for the densities of all SLWF stages (Tables 4-1 and 4-2). The reciprocal F_1 hybrids, LA 1340 x PI 127826 and LA 1340 X LA 386, did not differ significantly

Table 4-1. Mean density (cm^{-2}) of silverleaf whitefly on the adaxial surface of the 7th leaf node on plants of F_1 interspecific hybrids between L. pennellii, L. hirsutum, and L. esculentum , and the parents, evaluated in greenhouse multiple-choice bioassay for resistance to Bemisia argentifolii - Fall 1995.

Genotype	Taxon*	Number of plants	Eggs	Crawlers	2nd & 3rd instars	Pupae	Exuviae	Live adults	Dead adults
F_1(LA 1340 X PI 127826)	pen x typ	6	<0.1 e	<0.1 e	0 b	0 e	0 e	<0.1 a	0.2 b-e
F_1(LA 1340 X LA 386)	pen x typ	4	0 e	0 e	0 b	0 e	0 e	0 a	0.5 a-e
F_1(LA 1340 X LA1777)	pen x typ	3	0 e	0 e	0 b	0 e	0 e	<0.1 a	0.4 a-e
F_1(LA 1674 X LA 1353)	pen x typ	3	0 e	0 e	0 b	0 e	0 e	0 a	0.2 c-e
F_1(LA 1674 X LA1777)	pen x typ	3	0 e	0 e	0 b	0 e	0 e	<0.1 a	0.4 a-e
F_1(LA 2560 X PI 127826)	pen x typ	16	<0.1 e	0 e	0 b	0 e	0 e	<0.1 a	0.3 a-e
F_1(LA 2560 X LA 1353)	pen x typ	8	0 e	0 e	0 b	0 e	0 e	<0.1 a	0.9 a-c
F_1(LA 2560 X LA 1777)	pen x typ	8	0 e	0 e	0 b	0 e	0 e	<0.1 a	0.8 a-d
F_1(PI 127826 X LA 1370)	typ x pen	15	0 e	0 e	<0.1 b	0 e	0 e	<0.1 a	0.4 a-e
F_1(PI 127826 X LA 1674)	typ x pen	8	0 e	0 e	0 b	0 e	0 e	<0.1 a	0.3 a-e
F_1(LA 1353 X LA 1340)	typ x pen	16	0.1 e	<0.1 e	<0.1 b	0 e	0 e	<0.1 a	0.5 a-e
F_1(LA 1353 X LA 1674)	typ x pen	7	0 e	0 e	0 b	0 e	0 e	<0.1 a	0.5 a-e
F_1(LA 386 X LA 1340)	typ x pen	9	0 e	0 e	0 b	0 e	0 e	<0.1 a	0.6 a-e
F_1(LA 386 X LA 1674)	typ x pen	8	<0.1 e	0 e	0 b	0 e	0 e	0 a	0.2 c-e
F_1(Fla 7324 X LA 1340)	esc x pen	8	1.1 b-d	0.3 a-d	0.5 ab	0.3 a-d	0.1 c-e	0 a	0.2 c-e
F_1(Fla 7171 X LA 1340)	esc x pen	8	0.1 e	<0.1 de	<0.1 b	<0.1 e	<0.1 e	0 a	0.3 b-e
F_1(Fla 7324 X LA 1674)	esc x pen	6	0.8 b-d	0.3 a-c	0.2 b	0.1 a-e	0.1 b-e	<0.1 a	0.2 c-e
F_1(Fla 7324 X LA 2560)	esc x pen	8	1.2 a-c	0.2 b-e	0.6 ab	0.4 a	0.2 bc	<0.1 a	0.2 c-e
F_1(Fla 952050 X LA 2560)	esc x pen	7	0.5 c-e	0.1 c-e	0.1 b	<0.1 de	<0.1 e	<0.1 a	0.1 e

Table 4-1 -- continued

Genotype	Taxon*	Number of plants	Eggs	Crawlers	2nd & 3rd instars	Pupae	Exuviae	Live adults	Dead adults
F_1(Fla 7324 X LA 1777)	esc x typ	3	0.2 d-e	0.1 d-e	0.1 b	0.3 a-e	0 e	<0.1 a	<0.1 e
F_1(Fla 7324 X PI 127826)	esc x typ	8	0.6 b-e	0.2 b-e	0.2 b	0.1 b-e	<0.1 de	<0.1 a	<0.1 e
F_1(Fla 7324 X LA 386)	esc x typ	3	1.7 a	0.4 ab	0.1 b	0.1 c-e	<0.1 e	0 a	0.1 de
F_1(Fla 7324 X PI 126449)	esc x glab	8	0.6 b-e	0.2 b-e	0.4 ab	0.3 a-c	0.2 b-d	<0.1 a	0.1 e
F_1(Fla 7171 X PI 126449)	esc x glab	2	0.4 c-e	<0.1 e	<0.1 b	<0.1 c-e	0 e	0 a	<0.1 e
LA 1340	pen	8	0 e	0 e	0 b	0 e	0 e	<0.1 a	0.9 a
LA 1674	pen	8	0 e	0 e	0 b	0 e	0 e	0.1 a	0.5 a-e
LA 2560	pen	8	0 e	0 e	0 b	0 e	0 e	0 a	0.9 ab
PI 127826	typ	8	<0.1 e	0 e	0 b	<0.1 e	0 e	0 a	0.1 de
LA 1353	typ	8	0 e	0 e	0 b	0 e	0 e	<0.1 a	0.3 a-e
LA 386	typ	8	0 e	0 e	0 b	0 e	0 e	<0.1 a	0.3 a-e
LA 1777	typ	8	0 e	0 e	0 b	0 e	0 e	<0.1 a	0.4 a-e
PI 126449	glab	4	0.6 c-e	0.1 c-e	<0.1 b	0 e	0 e	0 a	<0.1 e
Fla 7324	esc	8	0.9 b-d	0.5 a	0.8 a	0.4 a	0.1 b-e	0 a	0.1 e
Fla 7171	esc	8	1.3 ab	0.4 ab	0.8 a	0.4 ab	0.2 bc	0 a	0.1 e
Fla 952050	esc	8	0.7 b-d	0.2 b-e	0.8 a	0.3 a-d	0.2 e	0.1 a	0.1 e

Means within a column followed by the same letter are not significantly different ($p \leq 0.05$) by Duncan's Multiple Range Test. Statistical analysis performed on square root transformed data, but means are original data.

* pen = L. pennellii; typ = L. trisutum f. typicum; glab = L. hirsutum f. glabratum; esc = L. esculentum.

Table 4-2. Mean density (cm^{-2}) of silverleaf whitefly on the abaxial surface of the 7th leaf node on plants of F$_1$ interspecific hybrids between L. pennellii, L. hirsutum, and L. esculentum , and the parents, evaluated in greenhouse multiple-choice bioassay for resistance to Bemisia argentifolii - Fall 1995.

Genotype	Taxon^{+}	Number of plants	Eggs	Crawlers	2nd & 3rd instars	Pupae	Exuviae	Live adults	Dead adults	Total* immatures
F$_1$(LA 1340 X PI 127826)	pen x typ	6	0.3 g	<0.1 f	<0.1 e	0 e	0 c	<0.1 a	0.1 bc	0.2 g
F$_1$(LA 1340 X LA 386)	pen x typ	4	0 g	0 f	0 e	0 e	0 c	<0.1 a	0.2 b	0 g
F$_1$(LA 1340 X LA1777)	pen x typ	3	0.7 g	<0.1 f	0 e	0 e	0 c	0 a	<0.1 bc	0.4 g
F$_1$(LA 1674 X LA 1353)	pen x typ	3	0 g	0 f	0 e	0 e	0 c	0 a	0.1 bc	0 g
F$_1$(LA 1674 X LA1777)	pen x typ	3	<0.1 g	0 f	0 e	0 e	0 c	<0.1 a	0.1 bc	<0.1 g
F$_1$(LA 2560 X PI 127826)	pen x typ	16	<0.1 g	0 f	0 e	<0.1 e	<0.1 c	0 a	0.1 bc	<0.1 g
F$_1$(LA 2560 X LA 1353)	pen x typ	8	<0.1 g	0 f	0 e	0 e	0 c	<0.1 a	0.2 bc	<0.1 g
F$_1$(LA 2560 X LA 1777)	pen x typ	8	0 g	0 f	0 e	0 e	0 c	0 a	0 bc	0.2 g
F$_1$(PI 127826 X LA 1370)	typ x pen	15	0.1 g	0 f	0 e	0 e	0 c	<0.1 a	0.1 bc	0.1 g
F$_1$(PI 127826 X LA 1674)	typ x pen	8	0 g	0 f	0 e	0 e	0 c	<0.1 a	0.2 bc	0 g
F$_1$(LA 1353 X LA 1340)	typ x pen	16	0.4 g	0.1 f	<0.1 e	0 e	0 c	<0.1 a	0.1 bc	0.3 g
F$_1$(LA 1353 X LA 1674)	typ x pen	7	0 g	0 f	0 e	0 e	0 c	<0.1 a	0.1 bc	0 g
F$_1$(LA 386 X LA 1340)	typ x pen	9	0 g	0 f	0 e	0 e	0 c	<0.1 a	0.2 bc	0 g
F$_1$(LA 386 X LA 1674)	typ x pen	8	0.1 g	0 f	0 e	0 e	0 c	0 a	0.2 bc	<0.1 g
F$_1$(FLA 7324 X LA1340)	esc x pen	8	5.3 c-g	1.7 c-f	2.8 a-e	1.0 c-e	0.7 bc	<0.1 a	0.1 bc	6.9 c-f
F$_1$(FLA 7171 X LA 1340)	esc x pen	8	0.1 g	<0.1 f	<0.1 e	<0.1 e	0.1 c	<0.1 a	0.1 bc	0.2 g
F$_1$(FLA 7324 X LA 1674)	esc x pen	6	12.3 ab	4.9 a	5.4 a	1.6 a-c	0.9 bc	0 a	<0.1 bc	13.3 ab
F$_1$(FLA 7324 X LA 2560)	esc x pen	8	7.3 a-e	2.5 b-e	4.3 a-d	1.3 c-e	0.4 c	0 a	<0.1 bc	9.2 a-e
F$_1$(FLA 952050 X LA 2560)	esc x pen	7	5.5 c-g	0.6 d-f	0.9 de	0.1 e	0.1 c	<0.1 a	<0.1 bc	4.0 d-f

Table 4-2 -- continued

Genotype	Taxon+	Number of plants	Eggs	Crawlers	2^{nd} & 3^{rd} instars	Pupae	Exuviae	Live adults	Dead adults	Total* immatures
F$_1$(FLA 7324 X LA 1777)	esc x typ	3	2.3 d-f	0.1 ef	1.6 de	0.2 de	0 c	0 a	<0.1 bc	2.4 d-f
F$_1$(FLA 7324 X PI 127826)	esc x typ	8	5.0 b-f	1.3 c-f	2.5 a-d	0.5 c-e	<0.1 c	<0.1 a	<0.1 c	5.3 b-e
F$_1$(FLA 7324 X LA 386)	esc x typ	3	9.8 a-c	4.3 ab	1.9 b-e	0.2 d-e	0 c	0 a	0 c	9.2 a-c
F$_1$(FLA 7324 X PI 126449)	esc x glab	8	7.9 a-d	2.1 a-d	3.4 a-d	0.8 c-e	0.3 c	<0.1 a	<0.1 bc	8.0 a-d
F$_1$(FLA 7171 X PI 126449)	esc x glab	2	10.6 a	1.2 c-f	1.3 c-e	0.5 c-e	<0.1 c	0 a	0 c	7.1 a-d
LA 1340	pen	8	0 g	0 f	0 e	0 e	0 c	<0.1 a	0.7 a	0 g
LA 1674	pen	8	<0.1 g	<0.1 f	<0.1 e	0 e	0 c	0 a	0.2 bc	<0.1 g
LA 2560	pen	8	<0.1 g	<0.1 f	<0.1 e	0 e	0 c	<0.1 a	0.2 bc	<0.1 g
PI 127826	typ	8	2.8 e-g	0.1 f	<0.1 e	0 e	0 c	0 a	<0.1 bc	1.5 fg
LA 1353	typ	8	0 g	0 f	0 e	0 e	0 c	0 a	0.1 bc	0 g
LA 386	typ	8	<0.1 g	0 f	0 e	0 e	0 c	0 a	0.1 bc	<0.1 g
LA 1777	typ	8	0.1 g	0.1 f	0 e	0 e	0 c	0 a	<0.1 bc	0.1 g
PI 126449	glab	4	3.3 c-g	0.2 ef	<0.1 e	0.1 e	0 c	0 a	<0.1 bc	2.1 e-g
FLA 7324	esc	8	5.7 a-e	3.1 a-c	4.6 a-c	2.7 a	2.2 a	<0.1 a	<0.1 bc	10.5 ab
FLA 7171	esc	8	9.2 ab	2.5 a-d	4.0 ab	2.3 a	1.1 ab	<0.1 a	<0.1 bc	11.0 a
FLA 952050	esc	8	4.6 b-f	1.0 c-f	3.2 a-d	1.3 b-d	0.6 bc	<0.1 a	<0.1 bc	6.4 a-e

Means within a column followed by the same letter are not significantly different ($p \leq 0.05$) by Duncan's Multiple Range Test. Statistical analysis performed on square root transformed data but means are original data.

+ pen = L. pennellii; typ = L. trisutum f. typicum; glab = L. hirsutum f. glabratum; esc = L. esculentum.

* Total immatures +exuviae averaged over total leaf surface area.

in the density of all whitefly stages. The difference between F_1(Fla 7324 X LA 1340) and F_1(7171 X LA 1340) for the total whitefly immatures was significant (Table 4-2).

Trichome density differences among accessions were highly significant (Appendix C-2) for both leaf surfaces. All the hybrids between L. pennellii and L. hirsutum had types I, IV, and VI, but no type Vs (Table 4-3). The hybrids of the cultivated tomato and the wild species possessed all four types of trichomes with the exception of one hybrid of PI 127826, which did not have type IV on any surface of the leaves, and one hybrid of PI 126449, which did not have type I and type IV on either leaf surface. Generally, hybrids of the cultivated tomato with either L. pennellii or L. hirsutum, had lower densities of type I and IV than the wild species parents, and the hybrids between L. pennellii and L. hirsutum.

Spearman correlation coefficients (r) were calculated between trichome densities and whitefly density measurements. The coefficients were generally low and were similar in magnitude and level of significance for both leaf surfaces (Table 4-4). Correlation of the density of live adults and any of the trichome densities was always low and either non-significant or had a marginal level of significance. In general, densities of type I and IV trichomess were negatively and significantly correlated with the density of whitefly immatures. Type VI density correlations with the immatures were in all cases positive and quite low but significant. For type V density, the correlation was positive and significant for all immatures, low but significant for dead adults on either leaf surface, and non-significant for live adults on the abaxial surface.

Table 4-3. Mean trichome density (cm^{-2}) for the 5th leaf node on plants of F$_1$ interspecific hybrids between L. pennellii, L. hirsutum, and L. esculentum, and the parents, evaluated in greenhouse multiple-choice bioassay for resistance to Bemisia argentifolii - Fall 1995.

		Number	Trichome type							
		of	Adaxial surface				Abaxial surface			
Genotype	Taxon*	plants	I	IV	V	VI	I	IV	V	VI
F$_1$(LA 1340 X PI 127826)	pen x typ	6	23 c-h	530 b-e	0 f	47 l-n	20 b-d	606 c-e	0 g	55 e-j
F$_1$(LA 1340 X LA 386)	pen x typ	4	35 a-d	423 d-h	0 f	22 j-n	11 b-g	616 c-e	0 g	23 f-l
F$_1$(LA 1340 X LA1777)	pen x typ	3	23 d-i	497 c-f	0 f	53 h-m	10 b-g	573 d-g	0 g	57 c-h
F$_1$(LA 1674 X LA 1353)	pen x typ	3	32 b-f	340 e-j	0 f	75 f-i	20 b-e	478 e-i	0 g	96 c-e
F$_1$(LA 1674 X LA1777)	pen x typ	3	22 d-i	280 h-k	0 f	20 k-n	18 b-d	342 g-k	0 g	49 d-i
F$_1$(LA 2560 X PI 127826)	pen x typ	16	26 b-g	625 a-d	0 f	18 l-n	8 c-h	709 c-e	0 g	8 j-l
F$_1$(LA 2560 X LA 1353)	pen x typ	8	30 b-g	353 e-i	0 f	50 h-k	19 b-d	528 e-h	0 g	41 d-i
F$_1$(LA 2560 X LA 1777)	pen x typ	8	32 a-f	320 f-k	0 f	81 f-i	13 b-f	433 e-j	0 g	44 c-h
F$_1$(PI 127826 X LA 1370)	typ x pen	15	24 b-h	554 b-e	0 f	12 n	10 b-h	629 c-e	0 g	4 l
F$_1$(PI 127826 X LA 1674)	typ x pen	8	24 b-g	739 ab	0 f	13 mn	8 d-h	845 bc	0 g	7 l
F$_1$(LA 1353 X LA 1340)	typ x pen	16	36 a-c	280 h-l	0 f	72 f-i	21 bc	490 e-i	0 g	58 c-f
F$_1$(LA 1353 X LA 1674)	typ x pen	7	40 a-c	327 f-j	0 f	106 e-g	19 b-d	531 e-h	0 g	92 bc
F$_1$(LA 386 X LA 1340)	typ x pen	9	31 b-f	447 d-g	0 f	21 k-n	9 b-h	578 c-f	0 g	17 g-l
F$_1$(LA 386 X LA 1674)	typ x pen	8	19 d-i	411 d-h	0 f	32 j-n	5 f-i	598 c-e	0 g	17 h-l
F$_1$(FLA 7324 X LA1340)	esc x pen	8	7 i-l	161 lm	107 e	23 j-n	5 f-i	267 j-n	178 f	25 f-l
F$_1$(FLA 7171 X LA 1340)	esc x pen	8	7 i-l	175 kl	86 e	31 j-n	2 g-i	283 j-m	113 f	22 f-l
F$_1$(FLA 7324 X LA 1674)	esc x pen	6	16 f-i	174 kl	95 de	59 g-j	6 e-i	214 k-n	270 f	44 c-i
F$_1$(FLA 7324 X LA 2560)	esc x pen	8	14 g-j	181 kl	90 e	43 h-l	5 f-i	346 f-k	109 f	18 f-l
F$_1$(FLA 952050 X LA 2560)	esc x pen	7	10 h-k	220 j-l	77 e	42 h-l	4 f-i	370 h-k	151 f	26 f-k

Table 4-3 -- continued

		Number	Trichome type							
		of	Adaxial surface				Abaxial surface			
Genotype	Taxon*	plants	I	IV	V	VI	I	IV	V	VI
F_1(FLA 7324 X LA 1777)	esc x typ	3	10 i-l	35 n	211 c	123 d-f	1 hi	150 l-n	454 c-e	50 c-g
F_1(FLA 7324 X PI 127826)	esc x typ	8	1 lm	0 o	265 bc	58 g-j	1 i	0 o	585 bc	45 c-h
F_1(FLA 7324 X LA 386)	esc x typ	3	17 f-j	61 mn	313 ab	147 c-e	2 g-i	104 n	520 b-d	87 b-d
F_1(FLA 7324 X PI 126449)	esc x glab	8	13 g-j	36 no	189 c	160 b-e	6 f-i	126 n	494 c-e	82 b-d
F_1(FLA 7171 X PI 126449)	esc x glab	2	0 m	0 o	232 bc	79 f-h	0 i	0 o	667 ab	76 c-e
LA 1340	pen	8	11 g-i	189 i-l	0 f	12 l-n	8 b-h	273 i-k	0 g	7 j-l
LA 1674	pen	8	13 g-i	198 i-l	0 f	23 j-n	5 e-i	204 k-n	0 g	9 i-l
LA 2560	pen	8	3 j-m	234 h-l	0 f	14 l-n	1 hi	303 i-k	0 g	5 kl
PI 127826	typ	8	17 f-i	420 d-h	0 f	240 a	5 f-i	838 b-d	0 g	185 a
LA 1353	typ	8	53 a	605 a-d	0 f	231 ab	38 a	1550 a	0 g	217 a
LA 386	typ	8	26 b-g	690 a-c	0 f	148 c-e	8 b-h	1070 b	0 g	99 b-d
LA 1777	typ	8	44 b	834 a	0 f	220 a-c	20 b	1067 b	0 g	226 a
PI 126449	glab	4	2 k-m	149 l	0 f	68 f-i	1 hi	537 d-g	0 g	139 ab
FLA 7324	esc	8	0 m	0 o	318 ab	176 a-d	0 i	0 o	905 a	41 d-i
FLA 7171	esc	8	0 m	0 o	379 a	110 e-g	0 i	0 o	886 a	25 f-l
FLA 952050	esc	8	0 m	0 o	195 c	154 b-e	0 i	0 o	702 ab	49 c-h

Means within a column followed by the same letter are not significantly different ($p \leq 0.05$) by Duncan's Multiple Range Test. Statistical analysis performed on square root transformed data but means are original data.

* pen = L. pennellii; typ = L. trisutum f. typicum; glab = L. hirsutum f. glabratum; esc = L. esculentum.

Table 4-4. Correlation (r) between the densities of B. argentifolii lifestages on the 7th leaf node and the density of trichome types on the 5th leaf node of F_1 interspecific hybrids between L. pennellii, L. hirsutum, and L. esculentum, and the parents evaluated in greenhouse multiple-choice bioassay for resistance to the silverleaf whitefly - Fall 1995.

Whitefly lifestage	Trichome type and leaf surface							
	Adaxial				Abaxial			
and leaf surface	I	IV	V	VI	I	IV	V	VI
Adaxial								
Eggs	-0.55***	-0.67***	0.78***	0.29***	-0.56***	-0.64***	0.77***	0.16**
Crawlers	-0.50***	-0.63***	0.75***	0.27***	-0.52***	-0.61***	0.76***	0.13*
2nd & 3rd Instars	-0.50***	-0.65***	0.76***	0.30***	-0.52***	-0.63***	0.77***	0.15*
Pupae	-0.46***	-0.62***	0.76***	0.30***	-0.50***	-0.60***	0.76***	0.16**
Exuviae	-0.61***	-0.56***	0.69***	0.26***	-0.46***	-0.53***	0.68***	0.10ns
Live Adults	0.05ns	0.05ns	-0.12*	-0.12*	0.11ns	0.03ns	-0.11ns	-0.08ns
Dead Adults	0.27***	0.34***	-0.45***	-0.27***	0.28***	0.31***	-0.464***	-0.19**
Abaxial								
Eggs	-0.61***	-0.69***	0.73***	0.30***	-0.57***	-0.63***	0.73***	0.20***
Crawlers	-0.57***	-0.67***	0.76***	0.32***	-0.57***	-0.64***	0.76***	0.19**
2nd & 3rd Instars	-0.57***	-0.71***	0.82***	0.30***	-0.58***	-0.69***	0.82***	0.16**
Pupae	-0.50***	-0.65***	0.80***	0.31***	-0.56***	-0.62***	0.80***	0.16**
Exuviae	-0.42***	-0.53***	0.64***	0.26***	-0.48***	-0.50***	0.64***	0.09ns
Live Adults	-0.11ns	-0.10ns	0.04ns	-0.11ns	-0.03ns	-0.11ns	0.02ns	-0.06ns
Dead Adults	0.18**	0.26***	-0.32***	-0.32***	0.21***	0.22***	-0.34***	-0.29***

260 observations were used in computing each correlation coefficient value.
ns, *,**,*** = Nonsignificant(ns) or significant at 0.05(*), 0.01(**), or 0.001(***) level.

Field Evaluation

The genotypes differed significantly in the density of total whitefly immatures on the abaxial leaf surface (Table 4-5). On the adaxial leaf surface, most plants had no whiteflies, and the genotypes did not differ significantly for any of the immature insect stages (Data not presented). On the abaxial surface, there were significant differences for the density of eggs, crawlers, and the 2nd and 3rd instar nymphs. All the hybrids between L. pennellii and L. hirsutum f. typicum were whitefly free. Hybrids of the cultivated tomato and LA 1340 were likewise whitefly free. The hybrid of the L. hirsutum f. glabratum accession PI 126449 crossed with the cultivated tomato had the highest whitefly density among the hybrids. Among the parents, only PI 126449 and LA 1777 had any whiteflies. The cultivated tomato lines had the highest whitefly density.

Differences among the genotypes were significant for the density of all trichome types (Table 4-6). Type V trichomes were exclusively found on the cultivated tomato and its hybrids with the wild accessions. The only exception was the hybrid of LA 1340 crossed with the tomato line 'Fla 7324'. There were no type IV trichomes on any leaf surface of the hybrid of the cross between PI 126449 and the cultivated tomato, whereas type I were not found on the adaxial leaf surface.

For the data taken from the abaxial leaf surface, type I and type IV trichomes had significant negative correlation coefficients with the density of eggs, crawlers, and 2nd & 3rd instar nymphs (Table 4-7). The densities of whiteflies on the adaxial leaf surfaces were low and generally were correlated at a lower degree. Because no pupae

Table 4-5. Mean density (cm^{-2}) of silverleaf whitefly on the abaxial surface of the 7th leaf node on plants of F_1 interspecific hybrids between L. pennellii, L. hirsutum, and L. esculentum , and the parents, evaluated in the field for resistance to Bemisia argentifolii - Fall 1995.

Genotype	Taxon$^+$	Number of plants	Eggs	Crawlers	Instars	Pupae	Exuviae	Live adults	Dead adults	Total* immatures
F_1(LA 1674 X LA 1353)	pen x typ	6	0 d	0 c	0 c	0	0	0	0	0 c
F_1(LA 2560 X PI 127826)	pen x typ	6	0 d	0 c	0 c	0	0	0	0	0 c
F_1(LA 2560 X LA 1353)	pen x typ	6	0 d	0 c	0 c	0	0	0	0	0 c
F_1(LA 2560 X LA 1777)	pen x typ	6	0 d	0 c	0 c	0	0	0	0	0 c
F_1(PI 127826 X LA 1340)	typ x pen	6	0 d	0 c	0 c	0	0	0	0	0 c
F_1(PI 127826 X LA 1674)	typ x pen	6	0 d	0 c	0 c	0	0	0	0	0 c
F_1(LA 1353 X LA 1340)	typ x pen	6	0 d	0 c	0 c	0	0	0	0	0 c
F_1(LA 1353 X LA 1674)	typ x pen	4	0 d	0 c	0 c	0	0	0	0	0 c
F_1(LA 386 X LA 1340)	typ x pen	6	0 d	0 c	0 c	0	0	0	0	0 c
F_1(LA 386 X LA 1674)	typ x pen	6	0 d	0 c	0 c	0	0	0	0	0 c
F_1(FLA 7324 X LA1340)	esc x pen	6	0 d	0 c	0 c	0	0	0	0	0 c
F_1(FLA 7171 X LA 1340)	esc x pen	6	0 d	0 c	0 c	0	0	0	0	0 c
F_1(FLA 7324 X LA 1674)	esc x pen	6	<0.1 d	<0.1 c	<0.1 c	0	0	0	<0.1	<0.1 c
F_1(FLA 7324 X LA 2560)	esc x pen	6	<0.1 cd	0 c	0 c	0	0	0	0	<0.1 c
F_1(FLA 952050 X LA 2560)	esc x pen	6	<0.1 d	0 c	0 c	0	0	0	0	<0.1 c
F_1(FLA 7171 X PI 127826)	esc x typ	4	0 c	0 c	0 c	0	0	0	0	0 c
F_1(FLA 7324 X PI 126449)	esc x typ	6	0.1 b	<0.1 c	<0.1 b	0	0	0	0	0.2 b
LA 1340	pen	6	0 d	0 c	0 c	0	0	0	0	0 c
LA 1674	pen	6	0 d	0 c	0 c	0	0	0	0	0 c

Table 4-5 -- continued

Genotype	Taxon$^+$	Number of plants	Eggs	Crawlers	Instars	Pupae	Exuviae	Live adults	Dead adults	Total* immatures
LA 2560	pen	6	0 d	0 c	0 c	0	0	0	0	0 c
PI 127826	typ	6	0 d	0 c	0 c	0	0	0	0	0 c
LA 1353	typ	6	0 d	0 c	0 c	0	0	0	0	0 c
LA 386	typ	6	0 d	0 c	0 c	0	0	0	0	0 c
LA 1777	typ	6	<0.1 d	0 c	0 c	0	0	0	0	<0.1 c
PI 126449	glab	6	<0.1 d	0 c	0 c	0	0	0	0	<0.1 c
FLA 7324	esc	6	0.2 ab	<0.1 a	<0.1 a	0	0	0	0	0.2 ab
FLA 7171	esc	6	0.3 a	<0.1 ab	<0.1 b	0	0	0	0	0.3 a
FLA 952050	esc	6	0.1 bc	<0.1 ab	<0.1 bc	0	0	0	0	0.2 b

Means within a column followed by the same letter are not significantly different ($p \leq 0.05$) by Duncan's Multiple Range Test.
Statistical analysis performed on square root transformed data, but means are original data.
+ pen = L. pennellii; typ = L. hirsutum f. typicum; glab = L. hirsutum f. glabratum; esc = L. esculentum.
* Total immatures + exuviae averaged over total leaf surface area.

Table 4-6. Mean trichome density (cm^{-2}) for the 5th leaf node on plants of F_1 interspecific hybrids between L. pennellii, L. hirsutum, and L. esculentum , and the parents, evaluated in the field for resistance to Bemisia argentifolii - Fall 1995.

Genotype	Taxon*	Number of plants	Trichome type Adaxial surface I	IV	V	VI	Abaxial surface I	IV	V	VI
F_1(LA 1674 X LA 1353)	pen x typ	6	23 a	329 b-e	0 d	22 e-h	10 a-f	369 e	0 e	34 e-g
F_1(LA 2560 X PI 127826)	pen x typ	6	16 a-f	403 b	0 d	25 a-h	11 a-e	528 b-e	0 e	8 fg
F_1(LA 2560 X LA 1353)	pen x typ	6	15 a-f	277 b-f	0 d	25 e-h	13 a-e	393 d-e	0 e	17 fg
F_1(LA 2560 X LA 1777)	pen x typ	6	20 a-c	272 b-f	0 d	54 b-g	12 a-e	376 e	0 e	19 fg
F_1(PI 127826 X LA 1340)	typ x pen	6	14 a-f	395 bc	0 d	23 e-h	10 a-f	438 c-e	0 e	9 fg
F_1(PI 127826 X LA 1674)	typ x pen	6	13 a-g	318 b-e	0 d	53 b-g	9 a-f	482 b-e	0 e	41 e-g
F_1(LA 1353 X LA 1340)	typ x pen	6	23 a	250 b-h	0 d	36 d-h	20 a	396 de	0 e	21 e-g
F_1(LA 1353 X LA 1674)	typ x pen	4	20 a-c	261 b-h	0 d	43 c-h	18 a-c	412 c-e	0 e	43 d-g
F_1(LA 386 X LA 1340)	typ x pen	6	16 a-f	326 b-e	0 d	26 e-h	8 b-f	434 c-e	0 e	7 fg
F_1(LA 386 X LA 1674)	typ x pen	6	11 c-g	230 c-h	0 d	51 b-g	11 a-e	346 e	0 e	25 e-g
F_1(FLA 7324 X LA1340)	esc x pen	6	11 c-g	221 d-h	4 d	35 e-h	6 d-f	539 a-e	0 e	6 fg
F_1(FLA 7171 X LA 1340)	esc x pen	6	12 b-g	269 b-f	0 d	11 h	7 c-f	597 a-d	0 e	4 g
F_1(FLA 7324 X LA 1674)	esc x pen	6	9 fg	95 i	130 b	65 b-f	7 c-f	196 f	256 c	8 fg
F_1(FLA 7324 X LA 2560)	esc x pen	6	10 e-g	160 f-i	63 c	21 f-h	5 d-g	406 c-e	67 c	8 fg
F_1(FLA 952050 X LA 2560)	esc x pen	6	9 e-g	261 b-g	6 d	17 f-h	5 d-g	471 c-e	11 e	7 fg
F_1(FLA 7324 X PI 126449)	esc x typ	6	0 h	0 j	204 ab	72 b-e	1 gh	0 g	877 a	27 e-g
F_1(FLA 7171 X PI 127826)	esc x typ	4	6 g	277 b-f	0 d	12 h	6 c-f	333 e	0 e	13 fg
LA 1340	pen	6	10 d-g	125 hi	0 d	14 h	4 e-h	184 f	0 e	10 fg
LA 1674	pen	6	8 g	137 g-i	0 d	15 h	3 f-h	190 f	0 e	5 g
LA 2560	pen	6	0 h	132 g-i	0 d	21 e-h	0 h	329 e	0 e	13 fg

Table 4-6 -- continued

Genotype	Taxon*	Number of plants	Trichome type: Adaxial surface I	IV	V	VI	Abaxial surface I	IV	V	VI
PI 127826	typ	6	10 d-g	405 b	0 d	120 ab	7 a-f	614 a-c	0 e	135 ab
LA 1353	typ	6	21 ab	438 b-d	0 d	84 b-d	19 ab	767 a	0 e	164 a
LA 386	typ	6	16 a-e	368 b-d	0 d	120 ab	13 a-e	680 ab	0 e	122 ab
LA 1777	typ	6	18 a-d	645 a	0 d	95 bc	15 a-d	623 a-c	0 e	110 a-c
PI 126449	glab	6	17 a-e	200 e-h	0 d	98 bc	5 e-h	686 ab	0 e	104 a-d
FLA 7324	esc	6	0 h	0 j	227 ab	182 a	0 h	0 g	736 ab	46 c-f
FLA 7171	esc	6	0 h	0 j	302 a	31 d-h	0 h	0 g	694 ab	27 e-g
FLA 952050	esc	6	0 h	0 j	224 ab	119 ab	0 h	0 g	671 b	163 b-e

Means within a column followed by the same letter are not significantly different ($p \leq 0.05$) by Duncan's Multiple Range Test.
Statistical analysis performed on square root transformed data but means are original data.
* pen = L. pennellii; typ = L. hirsutum f. typicum; glab = L. hirsutum f. glabratum; esc = L. esculentum.

and exuviae were observed on either surface, no correlation coefficients for these whitefly lifestages were computed.

Discussion

Greenhouse Evaluation

Most of the hybrids from the crosses between L. pennelli and L. hirsutum f. typicum were highly resistant. The most resistant genotypes had no immature lifestages of the insect. This category comprised the reciprocal F_1 hybrids PI 127826 X LA 1674, LA 1340 X LA 386, and LA 1674 X LA 1353, and their reciprocal crosses. The manner in which silverleaf whitefly resistance was expressed in reciprocal interspecific crosses of the wild Lycopersicon accessions could only be observed in the latter two crosses. Although other reciprocal crossings had been attempted (Muigai and Scott, 1996), they were unsuccessful; and, therefore, none were available for comparison. In the crosses of L. pennellii X L. hirsutum f. typicum investigated here all the F_1s were resistant to the silverleaf whitefly. The parents were selected for resistance, and it is not possible to discern the relative contribution of each parent in the hybrid resistance. The parents might be associated with different components of resistance. Moreover, the two parents might have a common component of resistance, but differ in the respective modes in which the componenet functions.

In two hybrids with LA 1340 as the pollen parent crossed with two L. esculentum lines ('Fla 7324' and 'Fla 7171'), the hybrids differed in the level of

Table 4-7. Correlation (r) between the densities of B. argentifolii lifestages on the 7th leaf node, and the density of trichome types on the 5th leaf node of F_1 interspecific hybrids between L. pennellii, L. hirsutum, and L. esculentum, and the parents evaluated in the field for resistance to the silverleaf whitefly - Fall 1995.

Whitefly lifestage	Trichome type and leaf surface							
	Adaxial				Abaxial			
and leaf surface	I	IV	V	VI	I	IV	V	VI
Adaxial								
Eggs	-.023**	-0.22**	0.21**	0.06ns	-0.09ns	-0.18**	0.21**	0.10ns
Crawlers	-0.16*	-0.14ns	0.13ns	0.08ns	0.04ns	-0.14ns	0.15*	0.07ns
2nd & 3rd Instars	-	-	-	-	-	-	-	-
Pupae	-	-	-	-	-	-	-	-
Exuviae	-	-	-	-	-	-	-	-
Live Adults	0.05ns	-0.07ns	0.10ns	0.07ns	0.10ns	0.04ns	-0.06ns	0.07ns
Dead Adults	-0.09ns	-0.09ns	0.13ns	0.02ns	-0.05ns	-0.09ns	0.14ns	-0.02ns
Abaxial								
Eggs	-0.53***	-0.58***	0.78***	0.32***	-0.51***	-0.52***	0.80***	0.22**
Crawlers	-0.49***	-0.50***	0.62***	0.29***	-0.41***	-0.50***	0.66***	0.18**
2nd & 3rd Instars	-0.45***	-0.46***	0.56***	0.29***	-0.37***	-0.46***	0.57***	0.22**
Pupae	-	-	-	-	-	-	-	-
Exuviae	-	-	-	-	-	-	-	-
Live Adults	-	-	-	-	-	-	-	-
Dead Adults	-0.09ns	-0.09ns	0.14ns	0.04ns	-0.09ns	-0.09ns	0.14ns	-0.10ns

169 observations were used in computing each correlation coefficient value.

ns,*,**,*** = Nonsignificant(ns) or significant at 0.05(*), 0.01(**), or 0.001(***) level.

- = not present.

resistance (Table 4-2). Except for this cross, the hybrids of the cultivated tomato with the wild genotypes were relatively more susceptible than wild X wild hybrids and the performance did not depend on whether the wild parent was either a L. pennellii or a L. hirsutum. Some hybrids were intermediate between the parents. Fery and Kennedy (1987) failed to find resistance to the tobacco hornworm in the F_1 and the F_2 of the crosses between PI 134417, and the cultivated tomato. They concluded that at least three recessive genes conditioned resistance to the tobacco hornworm in this accession. In their work, PI 127826 was a susceptible parent, whereas in the research reported here, this accession was one of the resistance sources. Earlier, PI 134417 was found to be moderately resistant to the SLWF (see Chapter 2).

The L. pennellii and L. hirsutum accessions did not have type V trichomes. This is in agreement with the description of trichomes in Lycopersicon by Luckwill (1943). The hybrids from crosses between these two species did not have type V trichomes. The hybrids between the wild Lycopersicon and the cultivated tomato had types I, IV, V, and VI (Table 4-3). Only the cross with L. hirsutum f. glabratum (PI 126449) did not have types I and IV. The cross of L. hirsutum f. typicum accession PI 127826 and the tomato (Fla 7171) did not have type V trichomes. In crosses of L. pennellii (PI 246502) and L. esculentum, Lemke and Mutschler (1984) reported that the presence of type IV trichomes was governed by 2-duplicate dominant epistatic genes, whereas the density of the same trichome type was inherited quantitatively with a high broad sense heritability. In F_1 hybrids of L. hirsutum (PI 251303) and L.

esculentum, Snyder and Carter (1985), observed trichome types I, IV, V, and VI among others.

The correlation coefficients between insect frequencies and the density of trichomes were negative and significant for the densities of type I and IV, and positive for densities of type V trichomes. In breeding for resistance to the whitefly, the density of trichomes might not be a reliable selection criterion, as it accounts for only a small proportion of the total variation for resistance found among genotypes.

Field Evaluation

The L. esculentum crosses with L. pennellii were as resistant as the crosses between two wild species. The one hybrid of the cultivated tomato and L. hirsutum f. typicum was resistant, whereas the one hybrid of tomato with L. hirsutum f. glabratum was as susceptible as the tomato parent. The field evaluation did not discriminate for resistance as powerfully as the greenhouse bioassay, despite the effort made to enhance the whitefly population by growing spreader rows of yellow squash plants. Therefore, greenhouse plants had a higher SLWF infestation than the field plants. Field grown plants tended to have a lower trichome density than greenhouse grown plants.

The correlation coefficients between trichome densities and the densities of whiteflies lifestages on adaxial leaf surfaces were mostly very low (Table 4-7). On the abaxial leaf surfaces, the densities of trichome types I and IV were negatively and significantly correlated to the densities of the eggs, crawlers, and the 2nd & 3rd instar nymphs.

The work reported here is a part of a research project the primary objective of which is to combine resistance components of wild Lycopersicon species that possess different profiles of resistance chemicals. Retention of useful levels of resistance when selecting for desirable horticultural characteristics has not been achieved in previous efforts. It is hoped that the enhanced resistance germplasm might transmit higher resistance even under selection for horticultural traits. The evaluations performed, especially the greenhouse bioassay, revealed that the hybrids of resistant wild Lycopersicon crossed with the susceptible cultivated tomato resulted in variable, but mostly susceptible progeny. The hybrids from L. pennelli X L. hirsutum crosses produced progenies that were highly resistant under the two test environments. These materials will now be advanced to the F_2 generation, where different levels of resistance might be expected as an outcome of recombination and the segregation of various genetic factors.

CHAPTER 5
EVALUATION OF F_2 INTERSPECIFIC HYBRID POPULATIONS OF LYCOPERSICON PENNELLII AND L. HIRSUTUM FOR RESISTANCE TO THE SILVERLEAF WHITEFLY

Introduction

The silverleaf whitefly (SLWF), Bemisia argentifollii Bellows & Perring, formerly sweetpotato whitefly biotype "B" (SPWF), is the major insect pest of tomato in south Florida (Schuster et al., 1996). The damage by this pest resulted in losses estimated at 500 million dollars to the United States agricultural industry in 1992 (Perring et al., 1993). The SLWF is difficult to control with insecticides because feeding and oviposition occur mainly on the lower surfaces of leaves (Sharaf, 1986), and because it develops resistance to insecticides applied for its control (Dittrich and Ernest, 1990; Stansly et al., 1991). Growing resistant cultivars would be advantageous because they are compatible with other components of integrated pest management, conserve the environment, and might slow the development of insecticide resistance because of reduced use of insecticides. However, no commercial cultivars with resistance to the whitefly are presently available (Schuster et al., 1996)

Wild Lycopersicon germplasm was screened and high levels of resistance to the SLWF were identified in several L. pennellii and L. hirsutum accessions (see Chapter 1). Previously, Berlinger et al. (1983), and Shevach-Urkin (1983), had attempted to use some of the same germplasm in breeding for resistance to the SPWF. No cultivar has been developed from those efforts because of the inability to transfer and retain effective levels of the resistance while eliminating the wild characteristics of the donor parents (J. W. Scott, pers. comm.). Barten et al. (1995) reported that the evaluation of the resistance in L. pennellii varied when tested by different methods and under different conditions. This might affect the efficiency of identifying resistance sources and resistant progenies when introgressing the resistance into the cultivated tomato.

With the objective to generate an alternative resistance source that could be used in breeding for resistance to the silverleaf whitefly, crosses were made between wild Lycopersicon accessions that had different profiles of resistance chemicals. In the F_1 generation, the hybrids were mainly resistant (see Chapter 4) and some of the resistant plants were selected to produce full-sib F_2 populations from which recombinants with multiple resistance chemicals could be recovered. Three F_2 full-sib populations were evaluated in a greenhouse multiple-choice bioassay, and the results are presented here. It is envisaged that the enhanced form of resistance produced from this research would enable the retention of usable levels of SLWF resistance in horticulturally acceptable tomato types.

Materials and Methods

Selection of F_1 Progenitors for Desirable F_2 Populations

Based on results of the greenhouse choice bioassay for resistance to the SLWF, 78 F_1 plants that had ratings of highly resistant or resistant, and 27 others that had a rating of moderately resistant, were selected to generate F_2 seeds (Appendix D). There were at least three plants from each of the 14 wild species hybrids that satisfied these criteria. However, among crosses between wild and cultivated species, only (Fla 7324 X LA 1340) and (Fla 7171 X LA 1340) satisfied the criteria, whereas in the other crosses, zero to two of the plants met the criteria. Therefore, 18 plants with a susceptible rating were also utilized for sib-mating in this latter category of crosses (Appendix D). The selected F_1 plants were cut back and after new shoots had regenerated, the plants were transplanted into greenhouse beds. When the plants bloomed during March-April 1996, the hybrids were at first manually self-pollinated; however, this was unsuccessful. Therefore, the hybrids were manually sib-mated with bulk pollen. Although the hybrids of the cultivated tomato were sib mated, these were not subsequently evaluated for resistance in the F_2 generation due to space limitations.

Evaluation of Resistance and Determination of Trichome Density of Greenhouse Grown F_2 Interspecific Hybrid Plants

Three full-sib populations: F_2(LA 1340 X PI 127826), F_2(LA 1340 X LA 386), and F_2(LA 2560 X LA 1777) were selected for evaluation of SLWF resistance. Because all the hybrids of L. pennellii X L. hirsutum were similarly resistant at the F_1

generation (see Chapter 4), these three populations were selected based on the differences of the chemical profiles of the L. hirsutum (Figure 5-1), and the L. pennellii parents (Figure 5-2). These two criteria were consistent with the objective to combine diverse resistance chemistries into one genotype, which would serve as a donor for resistance in future SLWF resistance breeding. Availability of greenhouse space was also another consideration in determining the sample size for this evaluation. All the F_1 progenitors of the three F_2 full-sib populations had been highly resistant to the SLWF.

For each of the three selected populations, approximately 200 seedlings were raised in two 128-celled (34 cm^3 cell size) styrofoam Speedling^R flats (Speedling Hort. Prod. Div., Sun City, Florida) containing Jiffy^R soil mix. At the fourth leaf stage, 120 plants were chosen randomly and transplanted into 1500 cm^3 pots containing a standard soil mixture. Twelve seedlings of tomato cv. Solar Set also were transplanted into pots. From each of the wild species parental plants, which had been maintained in the greenhouse, six cuttings were made and rooted under intermittent mist. Similarly, six cuttings were made from each of the progenitor F_1 plants that had been maintained in the greenhouse beds. The young rooted cuttings were transplanted into 1500 cm^3 pots at the time of transplanting the other seedlings. In early July 1996, all the pots containing transplanted test plants were randomly placed on benches in three insect-proof and water-cooled greenhouses. Forty plants from each population were randomized on the bench in each of the greenhouse. Supplemental lighting was provided using 40-watt cool white fluorescent and 300-watt incandescent bulbs to

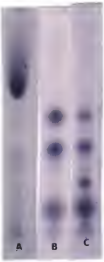


Figure 5-1. Thin layer chromatogram (C_8RP, vanillin stain) of Lycopersicon hirsutum f. typicum; accessions LA 1777 (lane A), PI 127826 (lane B), and LA 386 (lane C), that were used as parents in interspecific hybridization to combine different chemical profiles for resistance to Bemisia argentifolii.

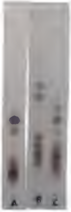


Figure 5-2. Thin layer chromatogram (C_8RP, orcinol stain) of Lycopersicon pennellii (Corr.) D'Arcy; accessions LA 2560 (lane A), LA 1340(a) (lane B), and LA 1340(b) (lane C), that were used as parents in interspecific hybridization to combine different chemical profiles for resistance to Bemisia argentifolii.

obtain a 16:8 (L:D)h photoperiod and 120-126 microeinsteins $m^{-2}s^{-1}$ (Li-cor Quantum meter, Model LI-185A, Lambda Inst. Corp., Lincoln, Neb.) light intensity. The lights were suspended about 30 cm above the plants and were raised as the plants grew. Temperature in the greenhouses ranged from 20-30 ^{0}C and the air was humid. The plants were watered as necessary. At 24 days after transplanting, the plants were mass infested with SLWF from a colony maintained in the laboratory on tomato cv. Florida Lanai.

The population density of whitefly adults, eggs, 1st-4th instars, and empty pupal cases (emerged adults) was determined at 30 days after mass infestation when the plants had approximately ten true leaves. Four leaflets proximal to the terminal leaflet on the leaves at nodes 5 and 7 (counting from the first unfolded leaf at the plant apex) were harvested using a pair of steel forceps to sever them from the plant and transfer them directly into a 15 cm diameter plastic petri dish. The samples were taken to the laboratory and whitefly adults, eggs, crawlers, and nymphs were counted on each leaflet surface for all the 8 leaflets using a dissecting microscope (Wild-Heerbrugg, Heerbrugg, Switzerland) set at a magnification of 12X. The density of type I, IV, V, and VI trichomes was determined from the average of counts made at four microscope fields on each surface of the pair of lateral leaflets proximal to the terminal leaflet on the leaf at node 5 described above. One of the leaflets was used to determine trichome density on the adaxial leaflet surface and the remaining one was used for the abaxial surface. The dissecting microscope (Wild-Heerbrugg, Heerbrugg, Switzerland) was set at a magnification of 50X for the counts. The fields of view, located in the interior middle section of the leaflet and with equal number on each side of the midvein, measured approximately 3.0 mm^2 each. The area of the leaflets used for insect counts, and trichome density determination was measured with a portable area meter (LI-COR model LI-3000, Lambda Instruments Corporation, Lincoln. Neb.).

Based on the number of immature whiteflies (eggs, 1st-4th instar nymphs, and exuviae) found on the sampled leaflets, plants were assigned a score of 1, 2, 3, 4 or 5, for none, 1-10, 11-50, 51-100, and more than 100 immatures, respectively. The distributions of resistance categories in the three F_2 populations, their F_1 progenitors, and the parental genotypes were compared. Spearman correlations between trichome densities and insect densities were calculated using the CORR procedure of the Statistical Analysis Systems (SAS Institute Inc., 1988).

Results

The frequency distributions of different resistance categories in the populations are in Figures 5-3 to 5-5. There was variation for resistance in the F_2 populations as shown by the distributions. The average densities of live and dead adults found on the leaves were comparable for all the genotypes. The F_1s and parental genotypes were resistant to the SLWF, which was consistent with previous observations (Appendix D).

Correlation coefficients between the densities of trichomes and the densities of whitefly lifestages among the F_2s were generally low (Table 5-1). For type I trichomes, the correlations were insignificant except those for the adaxial surface and pupae (r =

-0.12; $p<0.05$) and dead adults on the abaxial leaf surface ($r = 0.25$; $p<0.001$). Correlations between type IV and the densities of whitefly lifestages were negative and generally low but significant. Insignificant correlation was found between the densities of live and dead adults on both leaf surfaces and the density of exuviae on the abaxial surface relative to type IV trichomes on the adaxial leaf surface were. The type IV trichomes on the abaxial leaf surface had significant negative correlation with all SLWF lifestages on the abaxial leaf surface except for dead adults. The correlations between type VI trichome densities and the densities of whitefly lifestages were insignificant except for type VI trichomes and live adults on the abaxial leaf surface ($r = -0.13$; $p<0.05$). The correlations obtained for the combined F_2 populations (data not shown) were similar to those for the individual populations.

Discussion

The F_2 distributions of SLWF resistance classes segregated for susceptibility not found in the parents or the F_1 progenitors. The F_1s and the parental genotypes were resistant as expected because these materials were clones of plants previously selected for resistance. Examination of the characteristics of each F_2 population was undertaken because segregation of various genetic factors would be expected in this generation (Allard, 1960). There was variation among plants of the same population for the density of immature whitefly lifestages. The frequencies of plants with different resistance categories had a similar trend for the three populations (Figure 5-3 to 5-5), with approximately 80% of the plants in each population being highly resistant or resistant approximately 10% being moderately resistant and the rest of the plants either susceptible, or highly susceptible. The proportion of the combined susceptible and highly susceptible plants was 6%, 7%, and 11% for F_2(LA 1340 x LA 386), F_2(LA 1340 X PI 127826), and F_2(LA 2560 X LA 1777), respectively. The emergence of susceptible genotypes in the F_2 distributions indicates that recombination had occurred among the resistance factors from the different parents. Because the correlation coefficients between insect and trichome densities were low, it may be concluded that the differences in resistance are most likely associated with differences in chemical contents of exudates of glandular trichomes as opposed to the trichomes per se. The parents from which these populations originated were resistant and possessed different resistance chemistries. The TLC profiles of the exudates of glandular trichomes of the parental L. pennellii accessions (LA 1340 and LA 2560) revealed acylsugars of different compositions (Figure 5-2). The parental L. hirsutum f. typicum differed in the main chemical constituents of the exudates from their glandular trichomes (Figure 5-1). The main chemical constituents in the exudates of glandular trichomes of accession LA 1777 were sesquiterpene acids. Accession LA 386 and PI 127826 possessed sesquiterpene hydrocarbons and other sesquiterpenoids whose TLC characteristics were different from hydrocarbons. All the parents and their F_1s were found to possess antixenotic and antibiosis mechanisms of resistance to the SLWF (see Chapter 6 and 7). The chemical profiles of the F_2 plants were not processed; however, it is likely that

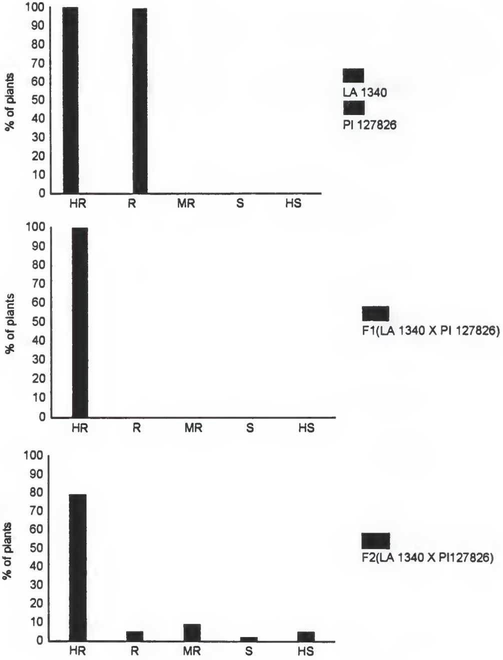


Figure 5-3. Frequencies of highly resistant (HR), resistant (R), moderately resistant (MR), susceptible (S), and highly susceptible (HS) plants, observed in greenhouse multiple-choice bioassay for resistance to silverleaf whitefly in F_2 full-sibs populations, and their F_1 progenitors, and respective L. pennellii (LA 1340, LA 2560), and L. hirsutum f. typicum (PI 127826, LA 386, LA 1777) parents - Summer 1996.
The F_1 progenitors and parents had been selected for resistance.
Resistance rating based on the number of whitefly immatures found on four leaflets of the leaf at node 7 counting from the plant apex.
HR = 0; R = 1-10; MR = 11-50; S = 51-100; HS = over 100.

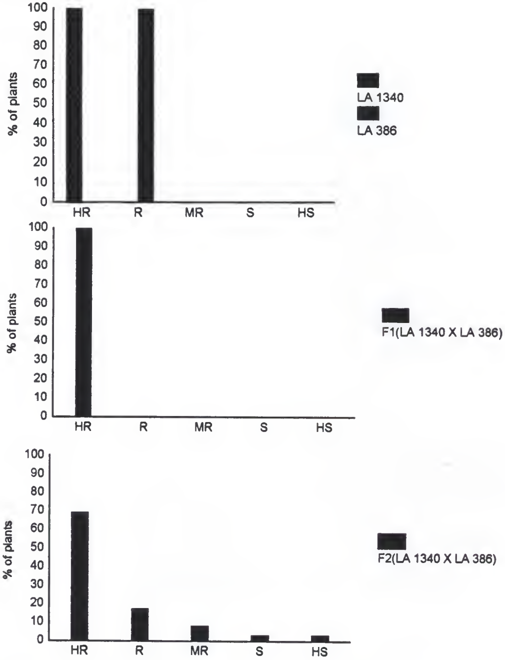


Figure 5-4. Frequencies of highly resistant (HR), resistant (R), moderately resistant (MR), susceptible (S), and highly susceptible (HS) plants, observed in greenhouse multiple-choice bioassay for resistance to silverleaf whitefly in F_2 full-sibs populations, and their F_1 progenitors, and respective L. pennellii (LA 1340, LA 2560), and L. hirsutum f. typicum (PI 127826, LA 386, LA 1777) parents - Summer 1996.
The F_1 progenitors and parents had been selected for resistance.
Resistance rating based on the number of whitefly immatures found on four leaflets of the leaf at node 7 counting from the plant apex.
HR = 0; R = 1-10; MR = 11-50; S = 51-100; HS = over 100.

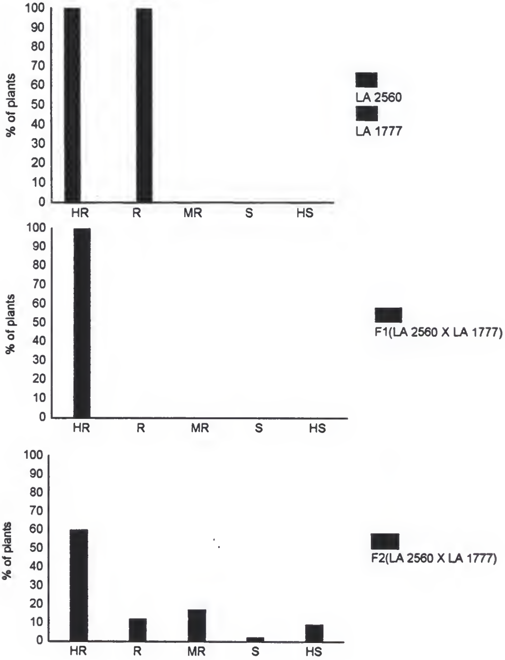


Figure 5-5. Frequencies of highly resistant (HR), resistant (R), moderately resistant (MR), susceptible (S), and highly susceptible (HS) plants, observed in greenhouse multiple-choice bioassay for resistance to silverleaf whitefly in F_2 full-sibs populations, and their F_1 progenitors, and respective L. pennellii (LA 1340, LA 2560), and L. hirsutum f. typicum (PI 127826, LA 386, LA 1777) parents - Summer 1996.
The F_1 progenitors and parents had been selected for resistance.
Resistance rating based on the number of whitefly immatures found on four leaflets of the leaf at node 7 counting from the plant apex.
HR = 0; R = 1-10; MR = 11-50; S = 51-100; HS = over 100.

Table 5-1. Correlation (r) between the densities of B. argentifolii lifestages on the 7th leaf node, and the density of trichome types on the 5th leaf node of full sib F_2 (LA 1340 X PI 127826), F_2 (LA 1340 X LA 1777), and F_2 (LA 2560 X LA 386) populations evaluated in greenhouse multiple-choice bioassay for resistance to the silverleaf whitefly - Summer 1996.

	Trichome type and leaf surface					
Whitefly lifestage	Adaxial			Abaxial		
and leaf surface	I	IV	VI	I	IV	VI
Adaxial						
Eggs	-0.06	-0.15**	-0.03	-0.01	-0.26***	-0.06
Crawlers	-0.06	-0.16**	-0.03	-0.01	-0.25***	-0.07
2nd & 3rd instars	0.02	-0.20***	0.04	0.06	-0.15**	<0.01
Pupae	-0.01	-0.14**	-0.02	0.07	-0.13*	-0.07
Exuviae	-0.05	-0.11*	-0.01	-0.01	-0.11*	-0.04
Live adults	-0.06	-0.05	-0.05	-0.03	-0.20***	-0.05
Dead adults	-0.09	-0.06	-0.04	-0.04	-0.16**	-0.03
Abaxial						
Eggs	-0.01	-0.17**	-0.05	0.03	-0.16**	-0.04
Crawlers	-0.05	-0.22***	-0.06	-0.04	-0.21***	-0.10
2nd & 3rd instars	-0.02	-0.08	0.03	<0.01	-0.21***	0.01
Pupae	-0.12*	-0.11*	-0.07	-0.03	-0.19**	-0.08
Exuviae	-0.03	-0.09	-0.02	-0.02	-0.16**	-0.05
Live adults	-0.06	-0.06	-0.05	-0.03	-0.20***	-0.13*
Dead adults	0.25***	0.08	0.02	0.10	0.02	-0.04

394 observations used in computing each correlation coefficient value.
Parents: L. pennellii accessions LA 1340 and LA 2560; L. hirsutum f typicum accessions PI 127826, LA 1777, and LA 386.
*,**, *** = significant at 0.05(*), 0.01(**), and 0.001(***) level.

the different resistance levels found in the F_2 plants represented different chemical combinations for resistance to the SLWF.

The L. pennellii parents in this and previous bioassays (see Chapters 2 & 6), were characterized by 1)the absence of immature whitefly lifestages and 2) the highest number of dead adults compared to other species. Few immature whitefly lifestages, as well as low numbers of dead adults were occasionally found on the L. hirsutum parents. In the bioassay for SLWF resistance in the F_2s, some plants in each F_2 population had a combination of immature lifestages and dead adults. This could be a further indication that the components of resistance inherent in the two parental species have been combined in some of the F_2s.

The correlation coefficients between the densities of whitefly lifestages and the trichome types were low (Table 5-1). The correlation coefficients between the density of type IV trichomes and the densities of whitefly immature lifestages were always negative and mostly significant. A similar phenomenon had been observed in the parents and F_1 generations. This indicates there is an association between the basis of the resistance and the type IV trichomes. However, because of the low correlation coefficients, the use of the density of type IV trichomes as a selection criterion for resistance to the silverleaf whitefly probably would not be an efficient means to identify resistant plants.

It has been speculated that the failure in previous breeding efforts to utilize whitefly resistance found in wild Lycopersicon germplasm might be attributed to the apparent tight linkage between the resistance and wild characteristics of the donor (J.

W. Scott, pers. comm.). The work reported here shows that recombination of resistance genes occurred and was manifested in segregants of different resistance levels. Selection for resistant genotypes with combined chemistries might be a way to obtain resistance with good horticultural type since not all genes from a single source would be needed. The linkage drag thus could be altered and possibly alleviated. Selection of resistant plants with combined chemistries for crossing with the cultivated tomato needs to be done to explore this possibility.

CHAPTER 6
EVALUATION OF THE MODALITIES OF RESISTANCE TO THE SILVERLEAF WHITEFLY IN SOME WILD LYCOPERSICON GERMPLASM

Introduction

Smith (1989) defines plant resistance to insects as those genetically inherited qualities that result in the plant of one cultivar or species being less damaged than a susceptible plant which lacks these qualities. This separates true resistance from pseudo-resistance and associational resistance.

Kogan and Ortman (1978) proposed the term antixenosis to replace non-preference, a term given by Painter (1951) for one of the mechanisms of plant resistance to insects. Antixenosis denotes the inability of a plant to serve as a host to an insect herbivore. According to Horber (1980), antixenosis is caused by the presence of morphological or chemical plant factors that adversely alter insect behavioral responses (orientation, contact, settling, feeding, and oviposition). The occurrence of antixenosis results in the selection of an alternative host. Antibiosis is another mechanism of plant resistance to insects (Horber, 1980). According to Painter (1951), antibiosis includes those adverse effects on the insect life history that result after an insect feeds on a resistant host plant. It is mediated by chemical and morphological plant defenses and is measured as death of immature insect stages, reduced growth rate, prolonged periods of development, reduced adult longevity and fecundity, and abnormal wandering and restlessness. Tolerance is another mechanism that is expressed when the plant continues to grow and reproduce in spite of supporting an insect population equal to that damaging a susceptible host. It is based on inherent genetic ability of a plant and often occurs in combination with antixenosis and antibiosis.

Plant morphological characters and plant allelochemicals are a possible basis for insect resistance (Smith, 1989; Smith et al., 1994). Morphological characters act as barriers to normal feeding and/or oviposition or habitation. Allelochemicals are either volatile or non-volatile allomones or kairomones which serve as cues in resistant genotypes.

Trichomes are one of the important morphological features of plant resistance to insects (Smith et al, 1994). The types of trichomes that occur in the genus Lycopersicon were described by Luckwill (1943). The main trichome differences between the cultivated tomato, Lycopersicon esculentum Mill., and the wild taxa L. hirsutum f. glabratum C. H. Mull., L. hirsutum f. typicum Humb. & Bonpl., and L. pennellii (Corr.) D'Arcy are the presence of type IV glandular trichomes exclusively in the wild species and the presence of type V non-glandular trichomes in the cultivated species. Shevach-Urkin (1983) reported that adults of the sweetpotato whitefly (SPWF) (Bemisia tabaci Gennadius) became entangled and were physically immobilized in the sticky exudate of glandular trichomes of the L. hirsutum f. glabratum accessions PI 134418 and PI 129157. Yorit (1986) reported similar results for accession LA 1777 of L. hirsutum f. typicum, but found no relationship between the level of entanglement and the number and type of glandular trichomes. Both investigators attributed the resistance in their materials to physical interference by the glandular trichomes. They found no correlation between resistance and the then recognized leaf volatile chemicals. Kisha (1981) observed that an L. esculentum accession with greater trichome density also had decreased oviposition and a higher ratio of SPWF females than males trapped on the leaves. On the basis of this observation, the conclusion was that trichomes could contribute to SPWF resistance in tomato.

In multiple-choice greenhouse bioassays (see Chapter 2) some accessions of L. pennellii, and L. hirsutum were found to have no, or fewer immatures of the silverleaf whitefly (SLWF), Bemisia argentifolii Bellows & Perring. and to have a higher number of entangled. Some accessions also had higher numbers of entangled live or dead adults than the susceptible tomato. The density of SLWF immatures was significantly and negatively correlated with the density of type IV trichomes, whose density had a significant positive correlation with the density of dead adult whitefly.

Bioassays were conducted with the objective to elucidate the mechanism(s) of resistance to the SLWF in resistant selections from some accessions of L. pennellii, L. hirsutum f. typicum, and L. hirsutum f. glabratum. The SLWF is a major pest of tomato in south Florida (Schuster etal., 1996) and is difficult to control with insecticides because feeding and oviposition occur mainly on the lower surface of leaves (Sharaf, 1986), and because it develops resistance to insecticides applied for its control (Dittrich and Ernest, 1990; Stansly et al., 1991). Consequently, growing resistant cultivars has the following advantages: they are compatible with other components of integrated pest management, conserve the environment, and slow the development of insecticide resistance. Resistant cultivars are not known to exist; therefore, information generated by this research should be useful in designing cultivar development strategies.

Materials and Methods

Eight plants representing the major chemical profiles that were found in L. pennellii, L. hirsutum f. glabratum, and L. hirsutum f. typicum in a previous multiple-choice experiment (see Chapter 2) were selected. Selected plants were cut back and 2 g of fertilizer (N:P:K; 20:20:20) dissolved in water at 4 g/l was applied to each potted plant, and multiple lateral shoots were allowed to develop. Cuttings were made from the shoots to produce clones of the selected plants. The cuttings were rooted under intermittent mist, then potted in 1500 cm^3 pots with regular potting media. The potted plants were placed on benches in a water-cooled, insect proof greenhouse and were watered as necessary. Supplemental lighting was provided using 40-watt cool white fluorescent and 300-watt incandescent light bulbs to obtain a 16:8 (L:D)h photoperiod and 120-126 microeinsteins $m^{-2}s^{-1}$ (LI-cor Quantum Meter, Model LI-185A, Lambda Inst. Corp., Lincoln, Neb.) light intensity. The lights were suspended above the plants and were raised as the plants grew. Temperature in the greenhouses ranged from 17-26 ^0C and the air remained humid. When the plants were at the 10-12 leaf stage, they were used in no-choice bioassays to determine the type(s) of resistance prevalent in these selected resistant plants. Plants of the cultivated tomato, L. esculentum cv. Solar Set, were grown from seed and served as the susceptible standard in this bioassay.

Settling Behavior and Mortality of the Adults

Leaf cages used in this bioassay were constructed from transparent extruded acrylic tubing. Each cage measured 12.5 cm in diameter and 15 cm long. One end of the cage was closed with organdy mesh held in place with an acrylic band that snugly slid over the outer wall of the cage. The opposite end had a slit (25 mm deep x 4 mm wide) carved in the wall of the tubular structure. During the bioassay, the leaf petiole of a plant attached leaf was mounted in this slit. To close this end, the edge of the cage fitted snugly into a circular groove carved on the surface of a transparent acrylic disk. The cage was mounted on a 15 cm X 15 cm square platform constructed from angle bar iron attached to a 150 cm tall steel pipe stand. The height of the platform was adjustable and extended perpendicularly to the stand so that the bioassay could be run using potted plants while they were still on the bench. The organdy covered and the acrylic covered ends were the functional floor and roof of the cage, respectively. A 3 mm diameter hole was drilled 25 mm from the organdy covered end in the side wall of the cage for introducing adult whiteflies into the test arena. A cork plug was used to close the hole during the bioassay.

To investigate the impact of the resistant germplasm on the settling behavior and mortality of adult SLWF, a no- choice bioassay was conducted using adult whiteflies from a laboratory colony maintained on tomato cv. Florida Lanai (Augustine et al., 1981). The terminal leaflet of the leaf at the 5th node of the resistant test plants, or the 3rd or 4th node of the susceptible control, was used for the bioassay. The leaflet was confined in the transparent cage mounted in an upright position. A ball of cotton wool was used to close the gap left between the mounted leaf petiole and the roof of the cage. A random sample of 20 mature SLWF females of unknown age was introduced into the cage. The numbers of whiteflies that were found on either the adaxial or the abaxial leaflet surface were recorded at 1, 2, 4, 12 and 24 hrs. After 24 hrs, the leaflet was severed from the plant while still mounted in the cage and brought to the laboratory, where it was carefully removed from the cage after removing the cotton wool plug at the slit in the wall of the cage. Eggs and dead whiteflies were counted with the aid of a dissecting microscope (Wild-Heerbrugg, Heerbrugg, Switzerland) set at 12X. Whiteflies were considered dead if they were immobile and no response was elicited when their abdomen was probed with a needle. There were 6 replications in a randomized complete block design. A different plant was used in each replication. There was one replication per block and the blocks were run sequentially in time. The plants were arranged randomly on the bench in the greenhouse. During each replication, the experiment proceeded in a sequence of 8 h in daylight, 8 h in darkness and finally, 8 h in daylight. During daylight periods, the supplemental lights were always on.

Oviposition Rate

The impact of the resistant genotypes on oviposition of SLWF was investigated in a no-choice bioassay in which 20 adult females were confined on the abaxial surface of the terminal leaflet on the leaf at the 5th node, using 2 cm diameter, 1.5 cm deep clip cages made of clear plastic with a nylon mesh covering both ends. The leaflet was positioned between the top 0.5 cm and the bottom 1.0 cm deep portions of the cage. Plants were placed randomly on the greenhouse bench in a randomized complete block design with 9 replications run sequentially in time. This bioassay was conducted in the same greenhouse and conditions described above. After 24 h, the leaflets were severed from the plant and brought to the laboratory where the eggs were counted as described previously.

To investigate the effects of trichome exudates on oviposition by the SLWF, the exudates were mechanically transferred onto the abaxial leaflet surface of an attached leaf of L. esculentum cv. Solar Set by gently appressing (temporarily bringing the leaflets together to transfer exudates and then separating them) an equivalent leaflet surface area of a resistant test wild genotype. The 'Solar Set' plants were raised in plastic pots in the greenhouse, and were used when they had 8-10 fully expanded leaves. Fully expanded terminal, or lateral leaflets on leaves at nodes 3 and 4, counting from the apex of the plant, were used for the bioassay. The four lateral leaflets proximal to the terminal one, on the leaves at node 5, counting from the apex of the resistant wild genotypes, were used in this bioassay. The leaflet of the resistant test genotype was severed using a pair of forceps, and the abaxial surface appressed gently, onto the abaxial surface of 'Solar Set' leaflet. As a control, the abaxial surface of a detached leaflet of 'Solar Set', was appressed onto the abaxial surface of the leaflet of the same cultivar. The no choice bioassay was conducted by confining 20 adult SLWF females of an unknown age on the treated leaflet surface using 2 cm diameter clip cages. After 24 hrs, the leaflets were detached and brought to the laboratory where eggs were counted as described above. The experiment was repeated 9 times in a randomized complete block design. The blocking was in time.

Oviposition, adult settling at 24 h, and adult SLWF mortality data were transformed (square root of X) prior to analysis of variance (ANOVA) using PROC GLM (SAS Institute Inc., 1988), and means were separated by the Duncan's Multiple Range Test. The progressive adult settling data were analyzed with PROC GLM with the repeated measures statement.

Results

Settling Behavior and Mortality of Adults

Analysis of variance for the number of whiteflies found on the leaflet surfaces in the bioassay using acrylic leaf cages revealed significant effects for genotype (F = 11.04; df = 10,55; P<0.001), leaflet surface (F = 90.09; df = 1,55; P<0.001), hours (F = 40.37; df = 4,55; P<0.001), genotype X leaflet surface (F = 16.88; df = 10,55; P<0.001), and leaflet surface X hours (F = 3.37; df = 4,55; P=0.010), but not for genotype X hours (F = 1.38; df = 40,55; P=0.075), and genotype X surface X hours (F

= 1.20; df = 40,55; P=0.204). For the 'Solar Set', 83% and 10% of the whiteflies were found on the abaxial and adaxial surface, respectively. On average, 23% and 59% of the whiteflies were found on the abaxial and adaxial leaflet surface, respectively, for the resistant wild genotypes (Fig. 6). The total number of whiteflies found on the leaflets increased with time for all the tested genotypes; however, there was a leaflet surface differential. At 24 h, the percentage of total whiteflies that were found on the adaxial surface of the L. hirsutum f. glabratum accession PI 126449 was significantly lower than that for other wild genotypes (except LA 386), which did not differ among themselves (Table 6-1). Significant differences were found among genotypes for the percentage of whiteflies found on the abaxial surface. The differences between the percentage of whiteflies found on the abaxial and adaxial surface were significant for all the genotypes except PI 126449.

Differences in the number of eggs deposited on either leaflet surface were significant (Table 6-1). The L. hirsutum f. glabratum accession PI 126449 had significantly more eggs on the adaxial surface, whereas the susceptible L. esculentum cv. Solar Set had significantly more on the abaxial surface. The other genotypes did not differ in the number of eggs deposited on any leaflet surface. The cultivar 'Solar Set' differed significantly from the wild germplasm in that on its leaflets, eggs were predominantly deposited on the abaxial surface. Differences between the number of eggs deposited on the two leaflet surfaces were significant for PI 126449 and 'Solar Set', but not for the other genotypes. Genotypes differed significantly in adult SLWF mortality for both the adaxial and abaxial leaflet surfaces (Table 6-1). L. pennellii and

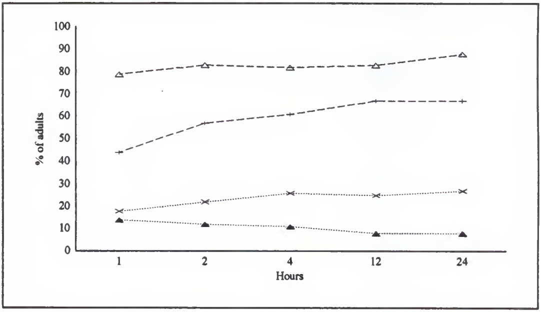


Figure 6. The percentage of adult Bemisia argentifolii found at 1, 2, 4, 12, and 24 hours on the abaxial (—▵—) and adaxial (—▴—) surface of leaflets of L. esculentum cv. Solar Set compared with the mean percentage found on the abaxial (—x—) and adaxial (—+—), leaflet surfaces of wild resistant Lycopersicon genotypes, in a no-choice greenhouse bioassay - October, 1995. Each data point is the mean of six observations for each genotype.

L. hirsutum f. typicum genotypes were not significantly different in whitefly mortality on the adaxial surface, but the two taxa had significantly higher mortality than the genotype from L. hirsutum f. glabratum accession PI 126449. 'Solar Set' had significantly less mortality than the wild Lycopersicon genotypes and had no dead whiteflies on the abaxial leaflet surface. The mortality differences between leaflet surfaces were significant for all the wild genotypes except PI 126449. Most of the whiteflies found on the leaflets of the wild genotypes were dead, whereas all of those found on accessions LA 1340 and LA 1353 were dead (Table 6-1).

Oviposition Rate

Differences among the genotypes were significant for the number of eggs deposited on the leaflets of the different genotypes (Table 6-2). No eggs were deposited on the L. pennelli genotypes tested, whereas the most were found on 'Solar Set'. There were significant differences among L. hirsutum genotypes; and, although they had significantly more eggs than the L. pennellii accessions, they had significantly fewer eggs than 'Solar Set'.

The transfer of the glandular trichome exudates of the resistant wild Lycopersicon germplasm to L. esculentum resulted in significantly fewer eggs deposited relative to Solar Set (Table 6-2). The number of eggs deposited differed significantly among the wild accessions. The exudates from L. pennellii genotypes resulted in the fewest eggs deposited but did not differ among themselves. Differences among L. hirsutum genotypes exudate effects were significant. Among the wild

Table 6-1. Bemisia argentifolii mean oviposition, adult settling, and mortality at 24 hours on leaflets of resistant wild Lycopersicon germplasm, and the cultivated tomato (L. esculentum) in a greenhouse no-choice bioassay using transparent acrylic leaf cages - October, 1995.

Taxa and accession number	Leaf surface and number of eggs: Adaxial	Abaxial	Difference	Leaf surface and adult settling (%): Adaxial	Abaxial	Difference	Leaf surface and adult mortality (%): Adaxial	Abaxial	Difference
L. pennellii									
LA 1340(a)	0 b	0 b	0	77 a	23 b-d	55†	77 a	23 ab	54†
LA 1340(b)	0 b	0 b	0	63 a	36 bc	27†	63 ab	36 a	27†
LA 1674	0 b	0 b	0	67 a	30 bc	37†	67 ab	29 ab	38†
LA 2560	1.5 b	0 b	1.5	68 a	21 cd	47†	66 ab	19 b	47†
L. hirsutum f. typicum									
LA 386	1.0 b	3.3 b	-2.3	58 ab	37 b	21†	55 b	36 a	19†
LA 1353	0 b	0 b	0	77 a	17 d	60	71 a	17 b	54†
LA 1777	1.0 b	3.8 b	-2.8	68 a	26 b-d	42†	67 ab	23 ab	44†
PI 127826	0.2 b	0 b	0.2	72 a	23 b-d	49†	66 ab	22 ab	44†
PI. 127827	1.2 b	4.5 b	-3.3	69 a	25 b-d	44†	66 ab	24 ab	42†
L. hirsutum f. glabratum									
PI 126449	12.7 a	5.3 b	7.3†	46 b	37 b	9	37 c	28 ab	9
L. esculentum									
Solar Set	3.0 b	67.7 a	-64.7†	8 c	88 a	-80†	1 d	0 c	1

Means within a column, followed by the same letter are not significantly different ($p<0.05$) by Duncan's Multiple Range Test.

Statistical analysis performed on square root transformed data, but means are original data.

Differences between means of adaxial and abaxial leaf surface for the number of eggs, settling, or mortality (%), followed by † are significant($p<0.05$), by the t-test.

Each mean is based on six observations.

Table 6-2. Bemisia argentifolii mean oviposition in 24 hours on leaflets of some resistant wild Lycopersicon germplasm, and on leaflets of a susceptible tomato cultivar whose leaflets had been appressed with glandular trichome exudates from the resistant wild germplasm; in a greenhouse no-choice bioassay using clip cages - November, 1995.

	Number of eggs deposited/leaflet	
Taxa and accession number	Leaflet of resistant wild genotype	Leaflet of cultivated tomato appressed with leaflet of resistant wild genotype
L. pennellii		
LA 1340	0 f	1 e
LA 1674	0 f	2 e
LA 2560	0 f	3 e
L. hirsutum f. typicum		
LA 386	10 c	15 d
LA 1353	3 e	24 bc
LA 1777	6 d	9 d
PI 127826	12 bc	22 c
L. hirsutum f. glabratum		
PI 126449	14 b	31 bc
L. esculentum		
Solar Set	90 a	92 a

Means within a column, followed by the same letter are not significantly different ($p<0.05$) by Duncan's Multiple Range Test.
Statistical analysis performed on square root transformed data, but means are original data.
Each mean is based on nine observations.

genotypes, the exudates of the L. hirsutum f. glabratum accession PI 126449 and the L. hirsutum f. typicum accessions LA 1353 and PI 127826 resulted in significantly greater oviposition compared to other wild genotypes.

Discussion

In the settling bioassay, significantly fewer SLWF were found on the leaflets of the resistant wild Lycopersicon genotypes than on 'Solar Set'. One hour after the bioassay started, 79% of the whiteflies introduced into the cage were found on the abaxial leaflet surface of 'Solar Set' and 14% were on the adaxial surface. In contrast, for the wild genotypes the range was 11-29% (mean 18%) on the abaxial surface, and 32-56% (mean 44%) on the adaxial surface. Over the duration of the bioassay, the number of whiteflies found on the leaflets increased for all the genotypes (Fig. 6), but increased differentially for the leaflet surfaces. At 24 hrs, 88% of the whiteflies were found on the abaxial and 8% on the adaxial surface of 'Solar Set', an increase for the former surface but a decrease for the latter leaflet surface. For the wild genotypes, the range at 24 hrs was from 17-37% (mean 27%) on the abaxial surface, and 46-77% (mean 67%) on the adaxial leaflet surface, an increase for both surfaces. Therefore, the salient modifications in the settling behavior of B. argentifollii on the leaflets of resistant plants selected from the wild accessions compared to the cultivated tomato were reduced settling in the initial period of the bioassay, reduced settling on the abaxial surface, and increased settling on the adaxial leaflet surface.

In the acrylic leaf cage bioassay, the main genotype effect and the genotype X leaflet surface interaction effect were significant for eggs deposited (Table 6-1). 'Solar Set' had significantly more eggs than the wild genotypes. The wild Lycopersicon genotypes did not differ significantly in the number of eggs on the abaxial surface; however, on the adaxial surface, the genotype from the accession PI 126449 had significantly more eggs than the other wild genotypes. Only the genotype from the accession PI 126449 and 'Solar Set' had significant differences in the number of eggs deposited on the two leaflet surfaces. On PI 126449 most of the eggs were on the adaxial surface, whereas on 'Solar set' most of the eggs were on the abaxial surface. The data show that, compared to 'Solar Set', oviposition on the leaflets of the resistant wild Lycopersicon was reduced by approximately 75% on the selection from PI 126449 of L. hirsutum f. glabratum and was reduced by between 90-100% on selections from the accessions of L. pennellii and L. hirsutum f. typicum.

Significant differences were found among genotypes in the adult whitefly mortality on either leaflet surface. Except for 'Solar Set', the mortality on the two leaflet surfaces differed significantly and the genotype X leaflet surface interaction also was significant (Table 6-1). In all instances, the larger number of dead whiteflies was found on the adaxial leaflet surface, and these differences were significant for all the wild genotypes except PI 126449. The dead whiteflies were found stuck onto the glandular trichome, most frequently by their tarsi, but in other instances by their wings, abdomen, or a combination of these insect body parts. These results corroborate those of Shevach-Urkin (1983) for some L. hirsutum f. glabratum accessions and

Yorit (1986) for some L. hirsutum f. typicum germplasm. None of the few dead whiteflies found on L. esculentum were stuck on trichomes, but were found recumbent on the leaflet surface.

All the assayed wild genotypes caused high adult whitefly mortality ranging from 77% to 100%. In contrast, only 1% whitefly mortality occurred on 'Solar Set'.

In the settling bioassay reported here, whitefly settling was reduced in the wild Lycopersicon germplasm treatments compared to the tomato. The number of adults on the leaflets increased as the exposure time increased. In the no-choice situation of this bioassay, the insect could not select an alternative host even if the substrate presented was inadequate. The scenario could fit Dethier's (1982) model for the influence of external and internal factors on insect investment behaviors. After some time without feeding, the internal excitatory cues due to starvation may have overwhelmed the external inhibitory cues from the resistant plant, resulting in the whitefly settling primarily to find food, whereupon, the whiteflies become entangled in the trichome exudates of the resistant plant. In the leaf cage experiments, significantly fewer eggs were deposited on the resistant wild genotypes compared to the tomato. When the glandular trichome exudates of the resistant genotypes were transferred onto the leaflets of the tomato, oviposition was reduced relative to the tomato, but the differences were smaller than in the intact leaflet bioassay (Table 6-2). It is suggested that this smaller reduction in oviposition on tomato leaflets on which glandular trichomes exudates had been mechanically transferred, relates to the efficiency of the distribution of the exudates. Reduced settling and oviposition can adversely affect the survival and propagation of the species. Therefore, on the basis of the reduced settling and oviposition, it is concluded that one of the mechanisms of resistance in the wild Lycopersicon genotypes assayed here is antixenosis. Because the transfer of exudates resulted in the transfer of the effects from the resistant to the susceptible genotype, the factors for the resistance could be located wholly, or in part, in the glandular trichome exudates.

In the acrylic leaf cage bioassay, the resistant genotypes had significantly more dead whiteflies than the tomato (Table 6-1). The dead whiteflies were found stuck on the glandular trichome exudates. No data on whitefly feeding on the resistant plants were collected. Moreover, whether the exudates have insecticidal activity other than the apparent physical entrapment and immobilization of the whiteflies is not discernible from the available data. However, the end result was the death of the whiteflies, which was caused by the characteristics of the resistant Lycopersicon genotypes. On this basis, it is concluded that one of the mechanisms of resistance in the wild Lycopersicon genotypes assayed is antibiosis, which can be effective on alighting adults and on eclosing adults.

The wild Lycopersicon genotypes reported in the above experiments apparently possess both antixenosis and antibiosis mechanisms of resistance to the silverleaf whitefly. The factors mediating both types of resistance are wholly or partly associated with the glandular trichomes of these genotypes.

CHAPTER 7

EFFECTS OF THE CHEMICAL CONSTITUENTS OF EXUDATES OF GLANDULAR TRICHOMES OF WILD LYCOPERSICON HIRSUTUM SPECIES ON THE SILVERLEAF WHITEFLY

Introduction

The silverleaf whitefly (SLWF), (Bemisia argentifolii Bellows & Perring) is a major pest of tomato (Lycopersicon esculentum Mill.) in south Florida (Schuster et al., 1996). The pest has been difficult to control with insecticides because it feeds and oviposits mainly on the abaxial leaf surfaces (Sharaf, 1986), and because the SLWF develops resistance to insecticides applied for its control (Dittrich and Ernest, 1990; Stansly et al., 1991). Host plant resistance could provide a means of managing the pest. Accessions of L. hirsutum f. glabratum and L. hirsutum f. typicum have been reported resistant to B. tabaci (Berlinger et al., 1983; Shevach-Urkin, 1983; Yorit, 1986) and B. argentifolii (see Chapter 2). The accessions demonstrated antixenosis and antibiosis types of resistance (see Chapter 6).

The volatile compounds that have been reported as major components of the exudates of glandular trichomes of L. hirsutum f. glabratum are the methyl ketones 2-tridecanone and 2-undecanone (Soost et al., 1968; Kennedy and Dimmock, 1983; Lin et al., 1987). The sesquiterpenes zingiberene and gamma-elemene (Weston et al., 1989; Carter et al., 1989), the sesquiterpene hydrocarbon acids bergamotenoic and santalenoic acids, which stimulated oviposition of tomato fruitworm (Coates et al., 1988), and 2,3-dihydrofarnesoic acid, which was repellent to mites (Snyder et al., 1993), have been reported as major volatiles in exudates of some accessions of L. hirsutum f. typicum. Kennedy and Dimmock (1983) associated mortality of Helicoverpa zea with 2-tridecanone.

Reported here, are the results of bioassays conducted with the purpose of elucidating the effects that selected chemical constituents of exudates of glandular trichomes of the resistant wild L. hirsutum have on the SLWF.

Materials and Methods

Chemical analogues of the main components of exudates of glandular trichomes of L. hirsutum were used in the bioassays. The methyl ketones 2-tridecanone, 2-undecanone, and 2-pentadecanone (all from Pfaltz & Bauer, Inc., Waterbury, CT), and ginger oil (Berje, Bloomfield, NJ), were evaluated for their repellency, fumigation, and residual toxicity on adult SLWF. Ginger oil, an extract of ginger (Zingiber officinale Roscoe) root, contains sesquiterpenes including zingiberene (66%), bisabolene (33%) and curcumene (1%) (Carter et al., 1989).

Repellency Bioassay

Insect cages (2 cm diameter) were used in a no-choice bioassay to evaluate the response of the SLWF to the leaflets of susceptible tomato cv. Solar Set, which had been sprayed with the test chemicals. The cages were constructed from 30 ml plastic diet cups (Fisher Scientific, Pittsburgh, PA). The cups were modified as follows: the bottom and side walls were partially removed to leave only two bands, each 0.5 cm wide at both ends of the cup, and to leave two 0.4 cm wide strips at positions directly opposite one another on the side wall. A piece of organdy was spread over the outside of the frame structure formed by the side strips and the bands. Another set of diet-cups were used to make 0.5 cm wide bands by cutting off the bottom to leave the rim of the cup for the band. One band was slipped over the organdy all the way to the wider end of the underlying frame thereby securing the organdy. When the open end was covered, the structure was a cage where whiteflies could be enclosed with optimized ventilation. To provide a port for introducing insects into the structure, a 0.5 cm diameter hole was made through the organdy and the frame, at a point where a side strip and the band joined at the bottom of the cage. The cage was mounted in a 4 cm hole in the middle of a 15 cm plastic petri-dish plate (Fisher Scientific, Pittsburgh, PA) such that the rim of the cage was flush with the bottom side of the top half of the petri-dish plate. The petri-dish plate with the cage was rested on a 15 cm X 15 cm square platform of angle-bar iron, which was mounted on a 150 cm tall stand constructed from a 25 mm diameter steel pipe. The height of the stand could be adjusted for the height of the plant.

A Potter Spray Tower (Burkard Manufacturing Co. Ltd, Rickmansworth, Herts, UK) was used to spray tomato leaflet surfaces with the designated quantities (ug cm^{-2}) of the chemical. The spray tower was calibrated using different volume:air pressure combinations to spray 95% ethanol on artificial surfaces mounted on the tower platform at 69 cm from the spray nozzle. The rate of deposition (ug cm^{-2}) of sprayed ethanol was determined using paper towel discs that were weighed before and after spraying known quantities of ethanol. The uniformity of coverage was determined by spraying clear polythene discs and visually inspecting droplet distribution. The optimum spray deposition:uniformity of coverage combination was realized when 6 ml were sprayed at 0.63 kg cm^{-2} air pressure, which deposited 3.36% of the total sprayed volume. With these sprayer conditions, the quantity (g) of chemical required for the treatment of a single experimental unit with a particular experimental concentration (ug cm^{-2}) was calculated by the equation:

[2.833 X experimental concentration in ug cm^{-2}] X 10^{-3}

This quantity of the chemical was dissolved in 6 ml of 95% ethanol and applied with the spray tower in the laboratory.

Tomato plants cv. Solar Set were grown in 1500 cm^3 pots in a greenhouse. When the plants had 10-12 leaves present, fully expanded leaflets of leaves in the middle third of the plant were selected for spray application. The attached leaflet to be treated was held by hand at the center of the spray tower platform with the abaxial surface facing upwards. After the spray operation was completed, the plants were left standing on a laboratory bench for 5-10 min for the ethanol to dry. The plants were then transferred to an insect rearing room maintained at 22 ^{0}C and 65% RH. The plants were placed on benches, and the spray treated leaflet was placed over the rim of the already mounted insect cage with the treated abaxial leaflet surface facing the inside of the cage. A 15 cm diameter transparent glass plate was then placed on the untreated adaxial surface of the leaflet so as to have an insect-proof compartment between the treated surface of the leaflet and the floor of the cage. Large midveins were avoided when mounting the leaflet in order to achieve better sealing of the cage.

Adult whiteflies for this bioassay were obtained from a laboratory colony maintained on tomato cv. Florida Lanai (Augustine et al., 1981). To elicit consistent response, the insects were aspirated from the plants and starved for 2-3 hr before they were used in the bioassay. Approximately 10 adult SLWF of unknown age were aspirated into the arena, and the introductory port was plugged with a cork stopper (Fisher Scientific, Pittsburgh, PA). After 10 min, the number of insects settled on the treated leaflet surface was recorded, using a flash light to illuminate the test arena. An exploratory test determined that 10 min was the minimum time required to obtain nearly 100% of the whiteflies settled on an ethanol treated leaflet.

For each test compound, exploratory tests established the concentration range encompassing no response (all settled on leaflet) and 100% response (all repelled). The concentrations (ug cm^{-2}) evaluated for 2-tridecanone were 4, 5, 6, 8, 9, and 10; for 2-undecanone 0.002, 0.004, 0.006, 0.008, 0.010, 0.012, and 0.014; and for ginger oil 0.005, 0.009, 0.018, 0.028, and 0.037. Ethanol-sprayed leaflets were used as controls. There were 4 replications for each concentration in a randomized complete block experiment.

Fumigation Bioassay

The apparatus for testing fumigation consisted of two parts: a fumigation chamber constructed from a 20 ml glass liquid scintillation vial and a fumes generator chamber constructed from a 3.7 ml clear glass vial (both vials from Fisher Scientific, Pittsburgh, PA). To clean the scintillation vials, they were soaked overnight in soapy water, rinsed in acetone, and air dried. The vials were then baked for at least 8 hr in a 150 °C oven to remove oils remainig from the manufacturing process. This vial cleansing procedure was adapted from the one used in insecticide resistance research (L. H. B. Kanga, pers. comm.). For the fume generator, the top of the screw cap of the 3.7 ml vial was removed leaving the threaded side walls intact. The chemical sample to be tested was deposited at the bottom of the small vial using a pipette or a spatula for liquid and solid samples, respectively. Organdy mesh was spread over the open end and neck of the vial and the cap screwed over it.

Test whiteflies were obtained from a laboratory colony maintained on tomato cv. Florida Lanai. In order to minimize injury to the insects when sampling, the plants were disturbed so that whiteflies flew and alighted on the wall of the cage. Approximately 20 adult SLWF of unknown age were then aspirated gently into the cleaned 20 ml scintillation vial. To ensure that only vigorous, healthy individuals were used for the bioassay, the vial was swiftly turned upside down and simultaneously brought up to an overhead light. The sides of the vial were gently tapped, whereupon, weak whiteflies were dislodged and dropped out of the vial. While the scintillation vial was held in this upside down position, the capped end of the 3.7 ml vial containing the test chemical was inserted into its neck forming an airtight union. In order to ensure that the chemical remained at the bottom of the fume generator, care was taken not to turn the small vial upside down. The completed fumigation apparatus was laid on its side atop a bench that was covered with a black cloth. Thus volatile chemicals would diffuse from the small vial into the larger vial and fumigation effects on the confined insects could be observed.

The fumigation bioassays were performed in an insect rearing room maintained at 23 ^{0}C and 65% RH. After 3 hr, the fumigation chamber was opened by pulling the two vials apart and the number of dead and live whiteflies recorded. Whiteflies were rated as dead when they lay on their ventral or dorsal sides, and rated alive when they were able to fly or to stand on their legs.

The chemicals 2-tridecanone and 2-pentadecanone were assayed in their natural solid state, whereas ginger oil and 2-undecanone were dissolved in 95% ethanol and 1 ul of the resulting solution applied to the fumigation unit. The ethanol was evaporated for 5 min before coupling the small vial fume generator to the large vial fumigation chamber. A fumigation unit in which no chemical was added served as a control. For the bioassay using 2-undecanone, a control treatment was prepared containing only 1 ul 95% ethanol applied and dried as above. For each test compound, exploratory tests established the concentration range encompassing no response (all alive) and 100% mortality. The concentrations (g/vial) evaluated for 2-tridecanone were 0.002,0.004, 0.006, 0.008, 0.010, 0.012, 0.014; for 2-undecanone (ug/vial) 0.0052, 0.0055, 0.0058, 0.0062, 0.0067, 0.0071, 0.0076, and 0.0083; and for ginger oil (ug/vial) 0.009, 0.046, 0.092, 0.459, 0.918, 1.377, 1.836, and 2.295. The test for each concentration of a chemical was replicated four times in a randomized complete block experiment.

Residual Toxicity Bioassay

All chemicals were evaluated for their residual toxicity using a vial technique. Different quantities of the test chemicals were dissolved in 200 ul of 95% ethanol and pipetted into 20 ml scintillation vials cleaned as above. The chemical was coated uniformly on the inside of the vials by rolling the vials at ambient temperature on a hot dog roller until they were dry, which occurred in 20 min. Approximately 20 adult whiteflies of unknown age were gently aspirated into each vial. Each vial was held upside down at an overhead light bulb, tapped lightly to dislodge and eliminate weak individuals, capped and laid on its side on a bench in an insect rearing room that was maintained at 23 ^{0}C and 65% RH. The vials were laid with their length parallel to fluorescent light bulbs in order to eliminate the tendency of some insects to settle on the untreated cap and upper neck portion of the vial. After 3 hr, each vial was uncapped and the number of dead and live insects was recorded. Insects that were lying either on their ventral or dorsal sides were counted as dead whereas the ones standing on their legs or flying were counted as alive. For each test compound, exploratory tests established the concentration range encompassing no response (all alive) and 100% mortality. Concentrations (ug cm^{-2}) evaluated for 2-tridecanone were 12.87, 13.80, 14.85, 15.80, 16.83, 17.80, 18.81, and 19.80; for ginger oil 0.031, 0.033, 0.035, 0.037, 0.039, 0.040, 0.042, 0.044, 0.046, and 0.048; and for 2-pentadecanone

6.56, 8.20, 9.84, 11.48, 13.12, 14.76, 16.40, and 18.04. The test for each concentration of a chemical was replicated four times in a randomized complete block experiment. Vials treated with 95% ethanol only were used as controls.

All data were subjected to probit analysis using the GENMOD procedure (SAS Institute Inc., 1988).

Results

The repellency concentrations (RC_{50}, RC_{90}), and fumigation and residual toxicity concentrations (LC_{50}, LC_{90}), for the different compounds, and the slopes of their respective concentration-response lines are shown in Table 7. In the repellency and fumigation bioassays, 2-pentadecanone did not have observable effects on the SLWF at any concentration that was practically possible to apply. The remaining three chemicals all demonstrated repellency and fumigation; however the RC_{50} and RC_{90} values for 2-tridecanone were approximately 200 and 500 times higher, respectively, than those of 2-undecanone or ginger oil. The LC_{50} and LC_{90} values of ginger oil for fumigation was about 100 times greater than that for 2-tridecanone and 2-undecanone.

Overall, the slopes were dissimilar for the chemical compounds evaluated for repellency (chi square = 206; p=0.0001); however, the slopes for 2-undecanone and ginger oil were more similar (chi square = 0.8; p=0.360) than the overall difference indicated. Slopes were different for the compounds evaluated in the fumigation bioassay (chi square =506; p=0.0001).

It was not possible to evaluate 2-undecanone for vial residual toxicity because at ambient temperature it is an oily liquid (Anon. 1995; Sax and Lewis, 1987) and whiteflies were entrapped immediately upon introduction into the vials. However, it was observed that the trapped whiteflies appeared to die almost immediately. The LC_{50} and LC_{90} values for 2-tridecanone and 2-pentadecanone were about 500 and 350 times higher, respectively, than those for ginger oil. The slopes for the evaluated methyl ketones were dissimilar (chi square =324; p=0.0001).

Discussion

All the compounds evaluated in the bioassays had some effects on the silverleaf whitefly adults (Table 7). Repellencies of 2-undecanone and ginger oil were comparable but were higher than for 2-tridecanone. Repellency increased with increasing concentration but at a slower rate for 2-tridecanone, as shown by the lower slope value. In the bioassays reported in Chapter 5, and in other reports (Shevach-Urkin, 1983; Yorit 1986), Bemisia spp. adults were found stuck and dead on the exudates of glandular trichomes of intact leaflets of L. hirsutum f. glabratum accessions known to contain 2-tridecanone and 2-undecanone, and on L. hirsutum f. typicum accessions containing sesquiterpene hydrocarbon compounds similar to the ones found in ginger oil. Repellency is one of the modes by which antixenosis could be manifested (Wiseman, 1985). The current finding supports the conclusion in Chapter 6 that antixenosis is one of the classes of resistance in Lycopersicon

Table 7. Effects of 2-tridecanone, 2-undecanone, 2-pentadecanone, and ginger oil (composed mainly of zingiberene) on repellency and survival of adult Bemisia argentifolii.

Bioassay	Parameter	2-tridecanone	2-undecanone	ginger oil	2-pentadecanone
Repellency[a]	Slope±SE	0.33±0.04	88.72±8.24	100.98±10.55	NA
	RC_{50}	6.53	0.03	0.01	NA
	95%FL	(6.05-7.01)	(0.029-0.031)	(0.009-0.011)	NA
	RC_{90}	10.47	0.05	0.02	NA
	95%FL	(9.51-11.43)	(0.048-0.052)	(0.017-0.023)	NA
	[c]χ^2	25.01	62.06	20.21	NA
	df	30	30	25	NA
Fumigation[b]	Slope±SE	275.18±17.44	1557.74±96.52	1.22±0.07	NA
	LC_{50}	0.008	0.005	0.89	NA
	95%FL	(0.0078-0.0082)	(0.0049-0.0051)	(0.85-0.94)	NA
	LC_{90}	0.013	0.006	1.94	NA
	95%FL	(0.0126-0.0134)	(0.0059-0.0061)	(1.86-2.02)	NA
	[c]χ^2	23.32	27.69	41.13	NA
	df	30	34	34	NA

Table 7 -- continued.

Residual[cd] toxicity	Slope±SE	0.48±0.03	-	229.28±12.89	0.36±0.02
	LC_{50}	16.42	-	0.03	10.72
	95%FL	(16.30-16.54)	-	(0.029-0.031)	(10.54-10.90)
	LC_{90}	19.11	-	0.04	14.22
	95%FL	(18.89-19.32)	-	(0.039-0.041)	(13.96-14.48)
	[e]χ^2	27.27	-	38.22	42.91
	df	34	-	42	34

a RC_{50} and RC_{90} values based on μg/cm² leaflet surface.

[b] LC_{50} and LC_{90} values based on amount per fumes generator: g (2-tridecanone); μg (2-undecanone); μg (ginger oil)

[c] LC_{50} and LC_{90} values based on μg per scintillation vial (internal surface area = 41.108 cm²).

[d] 2-undecanone was not assayed for residual toxicity because residues were sticky and entrapped adults at any dosage evaluated.

NA - no activity at any dosage evaluated.

[e] χ^2=Pearson chi-square for assessing goodness of fit to probit model.

df = (no. concentrations evaluated x no. replications) - 2.

FL - Fiducial limits for parameter estimates germplasm that possess these methyl ketones and sesquiterpene hydrocarbon compounds. For these compounds, the concentrations effecting the observed repellency are within the concentration ranges on leaves that have been reported for some wild Lycopersicon accessions (Kennedy and Dimmock, 1983; Weston et al., 1989).

The fumigation LC_{50} and LC_{90} values were lowest for 2-undecanone, followed by ginger oil and 2-tridecanone having the highest values. The conditions of this bioassay were different from the situation that occurs naturally because the fumigation chamber was airtight and the compounds were not contained in glandular trichomes. However, whiteflies that alight or become entangled in the exudates of trichomes of genotypes that possess these compounds would disturb the exudates and could become subject to fumigation. Fumigation is one of the modes through which the antibiosis could be manifested.

Residual toxicity LC_{50} and LC_{90} values were lower than the fumigation values for 2-tridecanone and ginger oil (Table 7). Ginger oil was about 500 times more toxic than 2-tridecanone. Since the scintillation vials were closed during the contact toxicity bioassay, the contact toxicity and fumigation effects might be confounded. Although 2-pentadecanone has not been reported as a major constituent of exudates of Lycopersicon species, it was included for comparison with the relatively shorter carbon chain methyl ketones. The results show that in the vial residual toxicity, it was about 25-50% less toxic than 2-tridecanone, but 200-300 times less toxic than ginger oil. Based on the trend of decreasing toxicity with increasing chain length, the toxicity of 2-pentadecanone should be less than that of 2-undecanone. These results are in agreement with those of Kennedy and Dimmock (1983) who reported 2-pentadecanone to be 20 and 9 times less toxic to tomato fruitworm, *H. zea*, than 2-tridecanone and 2-undecanone respectively.

All assayed compounds affected the SLWF in the three modes evaluated; however, 2-tridecanone was most effective as a contact poison; 2-undecanone was highly effective as a repellent and a fumigant; and 2-pentadecanone only had some contact toxicity effects. Two-undecanone also physically trapped SLWF adults even at the lowest concentration (0.002 ug/cm^2) evaluated. Ginger oil demonstrated repellency and residual toxicity, but lower fumigant toxicity relative to 2-undecanone but higher than 2-tridecanone. Only zingiberene and curcumene have been reported in the exudates of *L. hirsutum* f. *typicum* (Carter et al., 1989). The incorporation of 2-undecanone, 2-tridecanone and the sesquiterpene constituents of ginger oil into tomato germplasm could impart a high level of multi-modal resistance, which could be difficult for the SLWF to overcome.

CHAPTER 8
SUMMARY AND FUTURE PROSPECTS

Resistance to the silverleaf whitefly (SLWF), Bemisia argentifolii Bellows & Perring, was found in some accessions of Lycopersicon pennellii, L. hirsutum f. glabratum and L. hirsutum f. typicum, but not in L. pennellii var. puberulum and L. peruvianum. Intra-accession variation for resistance was observed. The field bioassay was less effective in discriminating resistance than the greenhouse bioassay, which included artificial infestation; however, out of 30 plants that were resistant in the greenhouse, only one was susceptible in the field. There was a low negative but significant correlation between the density of type IV glandular trichomes and the density of whiteflies. Therefore, density of glandular trichomes play a role in the observed resistance but cannot be a reliable selection criteria for resistance to the SLWF.

The chemical profiles revealed by Thin Layer Chromatography (TLC) differed among species, among forms of species, among accessions, and, in some cases, among plants within the same accession. Susceptible accessions had no visible bands or the bands were less dense. The profiles of L. pennellii accessions were characterized by acylsugar bands, and differences among and within accessions, when present, were in the number and polarity of the bands. L. hirsutum f. glabratum profiles were characterized by methyl ketone bands, and accessions differed in the presence or absence of the bands, whereas within accession differences were in the intensity of the bands. The TLC profiles of L. hirsutum f. typicum accessions mostly contained either typical sesquiterpene hydrocarbon bands or sesquiterpene hydrocarbon acid bands. However, the profile of L. hirsutum f. typicum accession LA 1353 contained bands that resembled methyl ketones, whereas the profiles of the L. hirsutum f. typicum accessions LA 386, PI 127826, and PI 127827 had some additional bands whose characteristics did not match those of previously observed components in this species. The isolation and identification of these components and investigation of their role in the resistance of these accessions might be useful.

Gas chromatography (GC) revealed chemical profiles that differed among species, among forms of L. hirsutum, among accessions and within accessions. L. hirsutum f. glabratum accessions generally had relatively early eluting components, whereas L. hirsutum f. typicum tended to have early and late eluting components. There were no major components in the profiles of L. peruvianum accessions. L. pennellii accessions were not analyzed by the GC method because their exudates are not volatile.

The profiles of exudates extracted using either hexane or methylene chloride were similar; however, extracts obtained using hexane were cleaner and produced acylsugar bands that were slightly better focused.

Based on the differences revealed by TLC and GC procedures, resistance appeared to be associated with the characteristics of chemical profiles. For the profile of any germplasm, absence of major components was invariably associated with susceptibility. Further, with acylsugars, plants with relatively less dense bands were relatively more susceptible. This was interpreted as indicating that the concentration of the acylsugars might be important in determining the level of resistance. Where the density of acylsugar bands appeared to be similar, genotype differences in the polarity and/or the number of acylsugar bands, were not associated with different levels of resistance. Therefore, the TLC data did not suggest an association of acylsugar quality with resistance. However, this does not preclude the influence of acylsugar quality on resistance because this observation was made at only the prevailing concentration during the bioassay. Future research could address this aspect.

Some plants of different wild Lycopersicon species that were resistant and represented the chemical diversity revealed by the TLC profiles, were selected to be recombined interspecifically to produce hybrid genotypes with combined chemistries and, hopefully, different mechanisms of resistance. Some resistant wild Lycopersicon were also crossed with susceptible cultivated tomato. Resistant F_1s were used to produce full sib F_2 families. The F_1s of crosses among resistant wild germplasm were generally resistant, whereas those with tomato were mainly susceptible, but with some resistant plants from crosses with L. pennellii, accession LA 1340. Profiles revealed by TLC were different from the parental profiles, and differed among crosses and within the F_1 plants of the same cross. The acylsugar bands appeared to overlay any profile characteristics that could be attributed to other chemical components. However, in general, the emergence of novel bands was common among the crosses. F_1s from the crosses between the tomato and L. hirsutum germplasm were mainly susceptible, and their profiles revealed by GC did not possess major components.

The acylsugar assay revealed that crosses differed in total quantity and the proportions of acylglucoses and acylsucroses. Compared to their respective L. pennellii parents, the proportions of acylsucroses and acylglucoses had changed, with most hybrids showing an elevated relative proportion of acylsucroses. There was significant positive correlation between the concentration of acylsugars and the density of trichome types I and IV.

There was segregation for resistance in the full sib F_2 populations. Approximately 80% of the plants were resistant, whereas the rest of the plants were either moderately resistant, susceptible or highly susceptible. The correlation between type IV trichomes and resistance was low and negative, but significant. It should be useful to extract trichome exudates of these plants and process their TLC profiles and acylsugar concentrations in order to account for differences in resistance, and possibly elucidate the fate of chemical components in the F_2 generation. The findings of the greenhouse bioassay need to be verified by either growing another sample of F_2 plants from the same populations or ideally but more difficult by growing clones of the greenhouse tested plants. The tested genotypes can be used in crosses as resistance donors to the tomato and subsequent selection be made based on the resistant TLC profile phenotypes and desirable horticultural characteristics. For conservation and future use, the genotypes need to be self-pollinated, where compatible, and if self-incompatible, matings done among plants with similar resistance within a population. It will be prudent to distribute some of the seeds to other national genetic resources repositories for long term conservation.

To investigate the modalities of resistance prevalent in the resistant parental genotypes, bioassays were conducted using plants representing the diversity of profiles revealed by TLC and GC analysis. One bioassay used intact leaflets whereas in a second bioassay the trichome exudates on the leaflets of the resistant genotypes were mechanically transferred onto the leaflet surface of the susceptible tomato by appressing. The bioassays with L. hirsutum revealed reduced settling and oviposition and increased mortality in the presence of the leaflets of the resistant genotypes or their trichome exudates. In a bioassay using pure chemical analogues of the known major components of the trichome exudates of L. hirsutum, it was found that although there were differences in the effectiveness of the chemicals, 2-undecanone, 2-tridecanone, and ginger oil (containing gingiberene, bisabolene, and curcumene) caused reduced settling, acted as fumigants, and had contact toxicity. These findings adequately supported the conclusion that antixenosis and antibiosis are functional classes of resistance in the wild L. hirsutum germplasm. The intact leaflets of resistant L. pennellii and their appressed trichome exudates had similar effects; however, practically no oviposition occurred in this case. On the basis of these findings it was concluded that antixenosis and antibiosis classes of resistance are operational in L. pennellii, but the mode of operation might be different from that found in the L.

hirsutum germplasm. These findings support the objective to combine different resistance chemistries from different Lycopersicon taxa to obtain better sources of resistance to the SLWF.

APPENDIX A
ANOVA RESULTS FOR SLWF AND TRICHOME DENSITY DATA FROM SCREENING OF WILD LYCOPERSICON GERMPLASM FOR RESISTANCE TO THE SLWF

A-1. ANOVA results for SLWF density on accessions evaluated in greenhouse choice bioassay - Fall 1994.

Whitefly lifestage	Leaf Surface	MSE	Fvalue	P>F
Eggs	Adaxial	0.47276	2.30	<0.001
	Abaxial	7.77806	4.24	<0.001
Crawlers	Adaxial	0.18881	3.02	<0.001
	Abaxial	1.63947	3.29	<0.001
2nd & 3rd Instars	Adaxial	0.06589	3.29	<0.001
	Abaxial	0.93687	3.61	<0.001
Pupae	Adaxial	0.00263	1.08	0.366
	Abaxial	0.14406	1.75	0.010
Exuviae	Adaxial	0.00058	1.46	0.059
	Abaxial	0.00008	1.09	0.346
Live Adults	Adaxial	0.00016	1.40	0.084
	Abaxial	0.00008	1.09	0.346
Dead Adults	Adaxial	0.22944	4.83	<0.001
	Abaxial	0.19008	6.46	<0.001
Total insects*	Both	194.25294	3.11	<0.001

F based on 32 and 247 df.
Solar Set, a susceptible commercial tomato cultivar, was used as a control.
* Total insects = eggs + crawlers + 2nd & 3rd Instars + pupae + exuviae

A-2. ANOVA results for trichome density types on accessions evaluated in greenhouse choice bioassay - Fall 1995.

Trichome*	Leaf Surface	MSE	Fvalue	P>F
Type IV	Adaxial	92.75991	20.88	<0.001
	Abaxial	146.96822	13.69	<0.001
Type V	Adaxial	76.45015	26.17	<0.001
	Abaxial	199.04261	26.86	<0.001
Type VI	Adaxial	39.71647	26.53	<0.001
	Abaxial	45.44065	45.35	<0.001

F based on 32 and 215 df.

Solar Set, a susceptible commercial tomato cultivar, was used as the control.

* Density of other trichome types not determined.

A-3. ANOVA results for SLWFdensity on selected plants evaluated in the field to verify resistance- Spring 1995.

Whitefly lifestage	Leaf Surface	MSE	Fvalue	P>F
Eggs	Adaxial	0.09144	1.17	0.278
	Abaxial	8.60378	4.81	<0.001
Crawlers	Adaxial	0.13636	1.57	0.050
	Abaxial	4.98123	4.87	<0.001
2^{nd} & 3^{rd} Instars	Adaxial	0.01768	0.92	0.599
	Abaxial	1.66584	8.19	<0.001
Pupae	Adaxial	-	-	-
	Abaxial	0.14104	13.55	<0.001
Exuviae	Adaxial	0.00114	0.38	0.999
	Abaxial	-	-	-
Live Adults	Adaxial	-	-	-
	Abaxial	-	-	-
Dead Adults	Adaxial	0.00546	0.71	0.878
	Abaxial	0.35829	0.38	0.999
Total insects*	Both	2521.42800	9.05	<0.001

F based on 37 and 75 df.

* Total insects = eggs + crawlers + 2^{nd} & 3^{rd} Instars + pupae + exuviae

A-4. ANOVA results for trichome density types on selected plants evaluated in the field to verify resistance - Spring 1995.

Trichome*	Leaf Surface	MSE	Fvalue	P>F
Type IV	Adaxial	565.91250	24.28	<0.001
	Abaxial	914.89590	36.95	<0.001
Type V	Adaxial	482.41780	59.26	<0.001
	Abaxial	1252.45750	51.38	<0.001
Type VI	Adaxial	158.40921	20.01	<0.001
	Abaxial	189.28323	10.42	<0.001

F based on 37 and 76 df.
Solar Set, a susceptible commercial tomato cultivar, was used as the control.
* Density of other trichome types not determined.

APPENDIX B
ANOVA FOR AMOUNT OF TRICHOME EXUDATES AND ACYL SUGAR IN F_1 INTERSPECIFIC HYBRIDS EVALUATED FOR RESISTANCE TO SLWF-FALL 1995

Trichome Exudate component	df Genotypes	df Error	MSE	F value	P>F
Crude extract	35	218	1.51551	9.49	<0.001
Acyl glucoses	35	140	4.13561	11.20	<0.001
Acyl sucroses	32	112	5.91641	9.13	<0.001
Total acyl sugars	25	112	4.63189	10.40	<0.001

APPENDIX C
ANOVA RESULTS FOR SLWF AND TRICHOME DENSITY DATA FROM F_1 INTERSPECIFIC HYBRIDS

C-1. ANOVA results for SLWF density on F_1 interspecific hybrids evaluated in greenhouse choice bioassay - Fall 1995.

Whitefly lifestage	Leaf Surface	MSE	Fvalue	P>F
Eggs	Adaxial	0.26961	5.81	<0.001
	Abaxial	4.50991	7.15	<0.001
Crawlers	Adaxial	0.03908	4.40	<0.001
	Abaxial	0.94141	4.93	<0.001
2nd & 3rd Instars	Adaxial	0.10663	4.25	<0.001
	Abaxial	1.77318	7.05	<0.001
Pupae	Adaxial	0.03879	3.64	<0.001
	Abaxial	0.50734	6.12	<0.001
Exuviae	Adaxial	0.01769	6.11	<0.001
	Abaxial	0.21918	4.14	<0.001
Live Adults	Adaxial	0.00049	0.71	0.886
	Abaxial	0.00008	0.99	0.486
Dead Adults	Adaxial	0.10718	2.67	<0.001
	Abaxial	0.02805	3.48	<0.001
Total insects*	Both	5.79189	10.02	<0.001

Square root transformation.
F based on 35 and 224 df.
* Total insects = eggs + crawlers + 2nd & 3rd Instars + pupae + exuviae

C-2. ANOVA results for trichome density on F_1 interspecific hybrids evaluated in greenhouse choice bioassay for resistance to SLWF - Fall 1995.

Trichome*	Leaf Surface	MSE	Fvalue	P>F
Type I	Adaxial	23.51208	14.61	<0.001
	Abaxial	12.30663	9.12	<0.001
Type IV	Adaxial	477.40790	38.87	<0.001
	Abaxial	66.763690	44.17	<0.001
Type V	Adaxial	270.95645	41.17	<0.001
	Abaxial	699.46610	49.02	<0.001
Type VI	Adaxial	108.68416	25.74	<0.001
	Abaxial	90.05385	17.96	<0.001

F based on 35 and 224 df.

* Density of other trichome types not determined.

APPENDIX D

SELECTION OF F_1 PROGENITORS OF F_2 FULL-SIBS-FALL, 1995.

	Plant Number																	
	1		2		3		4		5		6		7		8		Mean	
Pedigree	Score	Ext	Score	Ext	Score	Ext	Score	Ext	Score	Ext	Score	Ext	Score	Ext	Score	Ext	Score	Ext
LA 1353 X LA 1340 (a)	1*	530	2	632	1*	470	1*	426	1*	660	1	522	1	331	1	180	1.1	469
LA 1353 X LA 1340 (b)	3	482	1*	501	1*	566	5	241	1	444	1	654	1	370	1	568	1.8	478
PI 127826 X LA 1340	3	561	1*	573	1*	252	1	250	1*	901	1	833	1	696	1	347	1.2	552
LA 386 X LA 1340	1*	924	1*	1053	1*	523	1	697	1	133	1	877	1	665	1	649	1.0	690
PI 127826 X LA 1674	1*	865	1*	784	1	471	1*	520	1*	794	1	493	1	634	1	642	1.0	650
LA 386 X LA 1674	3	412	1*	606	2	675	1*	913	1*	672	1*	594	1	712	1	292	1.4	610
LA 1353 X LA 1674	1*	567	1	-	1*	751	1	554	1*	470	1	520	1	512	-	-	1.0	562
LA 1340 X PI 127826	4	490	1*	382	1*	501	1	610	1*	771	1*	721	-	-	-	-	1.5	579
LA 1340 X LA 386	1*	931	1*	641	1*	612	1	503	-	-	-	-	-	-	-	-	1.0	672
LA 1340 X LA 1777	2	1880	3	683	1*	421	1*	906	-	-	-	-	-	-	-	-	1.8	973
LA 1674 X LA 1353	1	-	1*	1041	1*	664	-	-	-	-	-	-	-	-	-	-	1.0	853
LA 1674 X LA 1777	-	-	1*	699	2	756	1*	634	-	-	-	-	-	-	-	-	1.3	696
LA 2560 X PI 127826	1*	624	2	998	2	527	1*	206	1*	674	1*	521	1*	376	1*	291	1.2	527
LA 2560 X LA 1353	2	523	1*	486	2	211	1*	543	-	-	1*	437	1*	512	1	367	1.2	440
LA 2560 X LA 1777	1*	525	1*	801	1*	592	1	321	1*	618	1*	755	1	471	1	466	1.0	569
Fla 7324 X LA 1340	1*	372	5	282	5	310	5	418	1*	573	5	251	1*	326	1*	344	3.0	360
Fla 7171 X LA 1340	3	490	1*	391	3	329	5	310	1*	677	1*	456	2	233	1*	357	2.1	405
Fla 7324 X LA 1674	5*	793	5*	610	5	277	5	205	1*	385	1*	358	-	-	-	-	3.7	438
Fla 7324 X LA 2560	3*	171	5*	713	5	401	5	103	1*	216	5	377	5	145	2*	290	3.9	302
Fla 952050 X LA 2560	5	166	3*	275	3*	244	5	181	2*	567	1*	255	5	98	3*	204	4.6	249
Fla 7324 X LA 1777	5*	241	5*	211	5*	206	-	-	5*	200	-	-	-	-	-	-	5.0	215
Fla 7324 X LA 386	5*	543	5*	256	-	-	-	-	3*	108	-	-	-	-	-	-	4.3	302

Appendix D -- continued

	Plant Number																	
	1		2		3		4		5		6		7		8		Mean	
Pedigree	Score	Ext	Score	Ext	Score	Ext	Score	Ext	Score	Ext	Score	Ext	Score	Ext	Score	Ext	Score	Ext
Fla 7324 X PI 127826	5	140	5	152	5*	306	5*	212	3*	140	5*	226	5*	192	5	121	4.8	186
Fla 7324 X PI 126449	5*	243	5	168	5*	271	5*	401	3*	267	2*	200	5	129	5	174	4.4	232
Fla 7171 X PI 126449	5*	199	5*	164	-	-	-	-	-	-		-	-	-	-	-	5.0	182
Fla 7324	5	491	5	213	5	267	5	465	5	440	5	219	5	205	5	213	5.0	314
Fla 7171	5	210	5	464	5	407	5	194	5	246	5	137	5	213	5	151	5.0	253
Fla 952050	5	286	5	262	5	410	5	378	5	413	5	-	5	187	5	311	5.0	321
LA 1340(pen)	1	833	1	776	1	1245	1	1433	1	1106	1	1294	1	896	1	1113	1.0	1087
LA 1674(pen)	1	1081	1	788	1	686	2	402	1	1167	1	4060	1	721	1	948	1.1	1232
LA 2560(pen)	1	1702	1	785	1	-	2	1166	1	1072	1	1314	1	537	1	565	1.1	1020
LA 386(typ)	1	572	2	593	2	546	1	345	1	537	1	395	1	399	1	291	1.2	460
LA 1353(typ)	1	1740	1	536	1	307	1	352	1	575	1	372	1	303	1	457	1.0	580
LA 1777(typ)	2	1193	3	299	1	654	3	547	1	863	1	431	1	412	1	449	1.6	606
PI 127826(typ)	5	364	4	473	3	309	3	234	1	546	1	301	1	254	1	277	2.4	345
PI 126449(glab)	4	286	3	422	5	434	2	458	-	-	-	-	-	-	-	-	3.5	400

* Plants utilized in sibmating to produce F_2 populations.

Score: 1=highly resistant; 2=resistant; 3=moderately resistant; 4=susceptible; 5=highly susceptible.

Pen=L. pennellii
typ=L. hirsutum f. typicum
glab=L. hirsutum f. glabratum
esc=L. esculentum
ext=hexane leaflet wash (µg/cm²)

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BIOGRAPHICAL SKETCH

Samuel Gaithuru Muigai was born in the Central province of Kenya on 1st August, 1953 to Alice Nyokabi and Suleiman Muigai. After his elementary education, he attended the Njiri and Kagumo high schools for his ordinary and advanced high school diplomas, respectively. In 1975, he started his degree studies at the faculty of agriculture and forestry of Makerere University in Uganda, but had to return to Nairobi University in Kenya where he completed the requirements for B.Sc.(Agriculture) in 1978. He, thereafter, worked for the Kenyan ministry of agriculture before enrolling for graduate studies at Nairobi University, and after submitting a thesis entitled "Characterization of the Ideal Bean (Phaseolus vulgaris L.) Genotype for Intercropping with Maize", was awarded M.Sc.(Plant Breeding) in 1991. During most of the 1980s, he worked for the Kenya Agricultural Research Institute where he was involved with the breeding of dry beans, and later green beans, before being appointed to perform research administration duties.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Mark J Bassett

Mark J. Bassett, Chair
Professor of Horticultural
Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

David J. Schuster, Cochair
Professor of Entomology
and Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

John W Scott

John W. Scott
Professor of Horticultural
Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

John C. Snyder
Associate Professor of
Horticulture and
Landscape Architecture
University of Kentucky

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Heather McAuslane

Heather J. McAuslane
Assistant Professor of
Entomology and Nematology

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

May, 1997

Jack L. Fry

Dean, College of Agriculture

Dean, Graduate School